JEUDI 27 JUIN 2013

SESSION 1 :

BIOMARQUEURS EMERGENTS

CONFERENCES PLENIERES

PERSPECTIVES THÉRANOSTIQUES DANS LE CANCER DU SEIN

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Auteurs présentant le résumé : Ivan Bièche

Résumé : La mise en évidence des altérations génétiques somatiques dans les tumeurs du sein a été une étape essentielle vers la compréhension des mécanismes moléculaires de l'oncogenèse mammaire. Ces altérations sont également potentiellement utilisables comme marqueurs en cancérologie clinique. En effet, certaines de ces altérations peuvent être utiles aux évaluations diagnostiques et pronostiques, à l'appréciation de la réponse au traitement et ouvrent actuellement la voie vers de nouvelles approches thérapeutiques.

Les altérations génétiques sont responsables de l'activation anormale d'oncogènes (gain de fonction) ou de l'inactivation de gènes suppresseurs de tumeurs (perte de fonction). Ces deux classes de gènes se distinguent par leur mécanisme d'action. Le mode d'action des oncogènes est considéré comme dominant, il suffit qu'un seul des 2 allèles soit activé pour qu'on observe un effet positif sur la tumeur. Tandis que le mode d'action des gènes suppresseurs de tumeurs est considéré comme récessif. Leur inactivation nécessite l'altération de leurs deux allèles.

La recherche dans le domaine des altérations génétiques du cancer du sein a vu récemment de profondes modifications concernant les stratégies méthodologiques. Le développement des technologies issues des puces à ADN (*microarrays, CGH-arrays*) au cours des années 2000-2010 a permis de changer d'échelle de travail en réalisant des analyses exhaustives de l'ensemble des gènes du génome humain. Plus récemment, le séquençage d'exomes a permis d'identifier l'ensemble des gènes mutées dans le cancer du sein. La puissance de ces méthodologies a permis d'identifier un nombre considérable d'événements moléculaires associés à la tumorigenèse mammaire.

Dans le cas du cancer du sein, les anomalies génétiques somatiques les plus fréquemment observées sont des amplifications d'ADN, principalement au niveau de proto-oncogènes ainsi que des délétions qui pourraient inactiver des gènes suppresseurs de cancer. Uniquement une vingtaine de gènes se sont révélés mutés à une fréquence supérieure à 1% dans les tumeurs du sein et plus particulièrement dans les tumeurs RE-positives. Les translocations chromosomiques ont été plus rarement détectées à ce jour. L'analyse exhaustive des ADN et ARN tumoraux par les technologies de séquençage de génomes ou de transcriptomes entiers devraient permettre rapidement de savoir si ce dernier mécanisme d'altération joue également un rôle important dans le développement du cancer du sein.

Amplifications et délétions d'ADN

Les amplifications d'ADN (principalement au niveau de proto-oncogènes) et les délétions (qui cibleraient des gènes suppresseurs de tumeurs) ont été recherchées à l'aide d'analyses pangénomes; dans un premier temps par cytogénétique, ensuite par hybridation génomique comparative (CGH pour *Comparative Genomic Hybridation ;* résolution de 10 Mb), puis actuellement par *CGH-array* (résolution de quelques Kb), et très prochainement par séquençage complet (résolution d'1 pb).

Ces techniques ont permis de caractériser une vingtaine de régions chromosomiques amplifiées et délétées dans les tumeurs du sein. Certaines de ces régions semblent associées à des oncogènes (*FGFR1*, *MYC*, *CCND1, ERBB2, CCNE1...*) ou à des gènes suppresseurs connus (*RB1,PTEN, TP53, CDKN2A , MAP2K4...*).

Les mutations ponctuelles

Malgré l'avancée significative apportée par les technologies de séquençage de nouvelle génération, ces techniques restent encore onéreuses pour envisager le séquençage à grande échelle du génome entier (3 Gb) d'un grand nombre de tumeurs. Une alternative, utilisée ces deux dernières années, est l'analyse de la séquence de l'exome, correspondant à l'ensemble des parties codantes du génome (1% du génome, ~30 Mb).

Les altérations du gène suppresseur *TP53* constituent l'événement génétique somatique le plus fréquent dans les tumeurs du sein. Outre des mutations du gène *TP53* retrouvées dans environ 40% des cas, on observe également des délétions de la région 17p13 dans les tumeurs du sein, en accord avec l'inactivation bi-allélique des gènes suppresseurs de cancer.

PIK3CA est l'oncogène qui montre la plus haute fréquence (environ 30%) de mutation "gain de fonction" dans le cancer du sein. *PIK3CA* code la sous unité catalytique p110▪ de la phosphatidyl inositol 3-kinase (PI3K). *PIK3CA* est principalement muté dans les exons 9 et 20, correspondant respectivement aux domaines « hélice alpha » (mutations E542K et E545K) et « kinase » (H1047R) de la protéine. Les mutations de *PIK3CA* sontt plus fréquentes dans les tumeurs RE▪-positive (30-40%) que dans les tumeurs RE▪-négative (10-20%). Finalement, les mutations de *PIK3CA* semblent responsables d'une résistance des tumeurs aux inhibiteurs des récepteurs membranaires à activité tyrosine kinase utilisés dans le cancer du sein : Trastuzumab et Lapatinib. Inversement, les mutations de PIK3CA pourrait constituer à l'avenir un marqueur de sensibilité aux inhibiteurs de la PI3K ou de mTOR.

On observe une exclusion mutuelle entre « mutation du gène *PIK3CA* » et « sous-expression du gène suppresseur *PTEN* ». Ce phénomène suggère que lorsque deux gènes appartiennent à une même voie de signalisation, l'altération d'un seul des deux gènes est suffisante pour que la voie de signalisation soit altérée.

Quatre autres gènes ont été montrés mutés avec une fréquence suprérieure à 5% dans le cancer du sein, c'est le cas du gène *CDH1* codant la Cadhérine E qui est muté plus particulièrement dans les carcinomes lobulaires invasifs et les gènes *GATA3*, *MAP3K1* et *MLL3* qui sont mutés plus particulièrement dans les tumeurs RE-positives.

Translocations chromosomiques et gènes de fusion.

Peu de translocations chromosomiques aboutissant à des gènes de fusion ont été identifiées à ce jour dans les carcinomes épithéliaux, ceci était dû en particulier à la difficulté d'étudier des caryotypes de carcinomes. Pourtant, l'identification de gènes de fusion, s'ils existent, dans le cancer du sein serait d'un grand intérêt clinique, en contribuant au diagnostic, au pronostic et à de nouvelles approches thérapeutiques. Deux exemples récents ont suggéré qu'il puisse exister de fréquentes translocations chromosomiques dans les carcinomes, au même titre que dans les hémopathies malignes et les sarcomes. Une délétion del(21)(q22.2;q22.3) ou une inversion chromosomique inv(21)(q22.2;q22.3) aboutit à un gène de fusion *TMPRSS2-ERG* dans 50% des tumeurs de la prostate, et une inversion chromosomique inv(2)(p23;p21) résulte en un gène de fusion *EML4-ALK* dans 5% des cancers du poumons. La présence du gène de fusion *EML4-ALK* est prédictive de la réponse au traitement par le *Crizotinib* (inhibiteur des récepteurs à activité tyrosine kinase Met, Ros1 et Alk).

Dans le cancer du sein, de rares translocations chromosomiquse ont été identifiées ces 10 dernières années. La translocation t(12;15)(p12;q26.1) qui fusionne les gènes *ETV6* et *NTRK3* est observée dans 85% des carcinomes sécrétoires du sein, une entité clinique très rare qui représente moins de 0.15% des tumeurs du sein. Des translocations impliquant les gènes *MAST, NOTCH, AKT3* et *FGFR2* ont également été identifiées, mais nécessitent confirmation par des études indépendantes.
De nouveaux gènes de fusion dans le cancer du sein sont actuellement activement recherchés par séquençage complet d'ADN ou d'ARN tumoraux.

Conclusion
L'avènement, ces 10 dernières années, des approches de *microarrays* ont permis les premières analyses pangénomiques (« Omiques ») des tumeurs du sein. De nombreuses altérations génétiques et un nombre plus restreint de voies de signalisation altérées (voies PI3K, NK-▪B, FGF…) caractéristiques des tumeurs du sein ont ainsi été identifiées. Cependant, bien que de nouvelles approches thérapeutiques aient été développées, la prise en charge des patientes atteintes de cancer du sein à l'aide de biomarqueurs n'a pas encore foncièrement changé. En effet, les applications cliniques en oncologie mammaire suite à l'identification l'altérations génétiques somatiques restent actuellement très limitées.
Les années 2012-2015 apporte et vont apporter une extraordinaire richesse de découvertes en cancérologie. Le séquençage intégral apportera une vision beaucoup plus complète et détaillée de l'ensemble des altérations génomiques d'une cellule cancéreuse. Le coût du séquençage d'un génome humain entier baisse de manière continue. Déjà inférieur à 10 000 €, il pourrait approcher à brève échéance les 1000 € puis très probablement 100 € à la fin de cette décennie. Le séquençage d'un gémome entier tumoral se substituera alors à la quasi-totalité des méthodes de génomique à grande échelle fondées sur l'hybridation (*FISH, CGH-array…*).
Cette prochaine décade verra apparaître les premières applications cliniques pour une patiente atteinte d'un cancer du sein basées sur les caractéristiques moléculaires de sa tumeur. Le séquençage des tumeurs permettra, par l'identification des gènes altérés dans la tumeur primitive puis dans les sous-clones cellulaires résistants responsables de métastases, de proposer de façon fiable la thérapie ciblée la plus appropriée à chaque tumeur et d'utiliser des biomarqueurs permettant une prise en charge personnalisée des patientes.
Enfin, des analyses non invasives des protéines et des acides nucléiques (ADN et ARN) au niveau du sang, permettront un suivi plus convivial du traitement et de la détection précoce des rechutes.

TUMOR MOLECULAR ANALYSIS BY A BLOOD TEST : APPLICATION TO KRAS MUTATIONS FROM CIRCULATING DNA IN COLORECTAL CANCER PATIENT.

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Auteur présentant le résumé : Alain R. THIERRY

Résumé : In the near future, the detection of circulating cell-free DNA (ccfDNA) could represent a technological breakthrough as diagnostic tool. Based upon our previous studies on release and structure of ccfDNA in plasma samples in a systematic way, we designed a refined and innovative method (Intplex) which simultaneously allows the determination of four parameters: the specific quantification of tumour-derived ccfDNA, the ccfDNA fragmentation index, point mutation detection and mutation load. Firstly, we could specifically and sensitively quantify the mutant and non-mutant tumour-derived ccfDNA in samples from patients with stage II to IV colorectal cancer (CRC), n=138, 100% success rate. In addition, we observed that, in these samples, tumour derived ccfDNA was more fragmented than ccfDNA from normal tissues. We carried out the first blinded prospective study to compare *KRAS* and *BRAF* mutational status data obtained from the analysis of tumour tissue with routine gold standard methods and of plasma DNA using our original method. The mutational status was determined by both methods in 106 patient samples. CcfDNA analysis showed 100% specificity and sensitivity for the *BRAF* V600E mutation. For the six tested *KRAS* point mutations, the method exhibited 100% specificity and 92% sensitivity with a concordance value of 96%. Mutation load, expressed as the proportion of mutant allele in ccfDNA, was highly variable (0.03% to 69%, median, 10.36%) among mutated samples (n=51 out of 138), showing very high inter-individual heterogeneity. Although Intplex is based on a classical method, this refined Allele Specific Q-PCR exhibit high performance as it enables single-copy detection of variant alleles down to a sensitivity of ≥ 0.005% mutant to WT ratio. These selectivity and detection limits are equivalent to the sophisticated Digital-based Q-PCR BEAming technology. The mean turnaround time is two days as compared to 28 days as recently reported for tumour-tissue analysis. In addition to its unprecedented sensitivity and specificity, this approach is easy, rapid and cost effective, and encourages non-invasive tumour molecular analysis potentially allowing diagnosis, prognosis and cancer patient follow-up, the detection of tumour growth, in parallel with currently available tests, or theranostics towards personalized medicine. Finally, in order to improve ccfDNA analysis, we decided to focus on preanalytical handling of samples and defined the first guideline for the translation of ccfDNA analysis in routine clinical laboratories.

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SESSION 2 :

NOUVELLES APPROCHES EN IMAGERIE ONCOLOGIQUE

CONFERENCES PLENIERES

ONCO-PHOTONICS FOR DIAGNOSIS: INTEREST OF INFRARED AND RAMAN MICRO-IMAGING

Auteurs et adresses : Olivier Piot, Cyril Gobinet; Jayakrupakar Nallala, Teddy Happillon, Ganesh Sockalingum, Pierre Jeannesson, Michel Manfait
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Auteur présentant le résumé: Olivier Piot

Résumé : Innovative diagnostic methods are the need of the hour that could complement the gold standard cyto-/histo-pathology for cancer diagnosis. In this perspective, biophotonic approach such as vibrational spectral micro-imaging (including infrared (IR) absorption and Raman scattering) is one of the candidate methods, as it provides spectral fingerprint of cell and tissue biochemistry in a non-destructive and label-free manner.

In this presentation, examples of application at cell and tissue levels will be given:
1/ the first example corresponds to a novel concept of IR spectral histopathology of paraffinized colon tissue arrays, with the aims to: identify spectral signatures specific of colon histological structures and exploit these signatures to develop a prediction model comprising potential diagnostic markers for rapid and automated colorectal cancer diagnosis. IR imaging associated to advanced statistical data processing enabled to identify important normal colonic characteristics like the well-differentiated normal epithelium, the peri-cryptal fibroblastic sheath (PCFS) corresponding to the basement membrane of colonic glands; and malignant characteristics like the loss of differentiation of normal epithelium, irregular PCFS, tumor-associated stroma. In addition, important features difficult to discern by conventional histopathology like tumor budding, tumor-stroma interphase, and minute inflammation, were easily identified by this methodology.
2/ the second example (IHMO ANR project) demonstrates the power of Raman micro-spectroscopy coupled with supervised classification algorithm such as Support Vector Machines for cell classification and more precisely for the diagnosis of chronic lymphocytic leukemia from blood smear. The originality of the approach consists in the development of a multimodal microscopy scanning platform that combines in a single machine a Raman micro-spectrometer and a multispectral imaging set-up.

Thus, via a single analysis, rapid molecular level diagnosis of the cell or tissue is obtained in a label-free and non-destructive manner. This study demonstrates the potential of vibrational spectral analysis combined with multivariate statistical data processing as a complementary tool to conventional cyto-/histo-pathology, for an automated and objective cancer diagnosis. In addition, remote fiber-based probes dedicated to real time measurements in clinics, are under development for interventional purposes during surgery.

NUCLEAR IMAGING – A TRANSLATIONAL TOOL IN ONCOLOGY DRUG DEVELOPMENT

Auteur présentant le résumé : Xavier Tizon, Oncodesign

Résumé : Preclinical imaging has over the last years shown its high potential to support and accelerate drug development in oncology, especially for targeted therapies. Across therapeutic areas, imaging endpoints are showing promise as quantifiable measures of efficacy and disease response. By giving quantitative readouts from live organisms, imaging helps to study biological processes that occur at the cellular and molecular level. Imaging is also unique in its ability to noninvasively assess the spatial and temporal heterogeneity of tumors, during their development or after a therapeutic intervention.
Several modalities exist, often routinely used in clinical settings, making them particularly relevant for translational research, using adapted animal models.

Nuclear imaging is among the most sensitive technique, allowing imaging procedures to be performed using only very low amounts of tracers, much below the pharmacologically active dose. Despite its drawbacks in terms of logistics and cost, it offers a vast library of tracers available to measure relevant pharmacological endpoints, some of them also available for clinical use. Moreover, it is now possible to label and image all types of drugs, from small molecules to antibodies, using carefully chosen isotopes and radiochemistry strategies.

In this talk, I will present the main principles behind nuclear imaging and explore, through examples, the different applications of this technique in preclinical and clinical pharmacology and oncology drug development. Most of these examples will be based on our experience through the development of the Pharm'image consortium, aiming at creating a multidisciplinary platform in Dijon to foster the evaluation of novel molecules and to encourage the emergence of pharmaco-imaging as an essential tool in drug development.

COMMUNICATIONS ORALES SELECTIONNEES :

SESSIONS 1, 2 ET STRATEGIES THERAPEUTIQUES

A POOR PROGNOSIS SUBTYPE OF HNSCC IS CONSISTENTLY OBSERVED ACROSSMETHYLOME, TRANSCRIPTOME AND MIRNOME ANALYSIS

Auteurs et adresses : Alain C. Jung1, Sylvie Job2, Sonia Ledrappier1, Christine Macabre1, Joseph Abecassis1, Aurélien de Reynies2, Bohdan Wasylyk3
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Auteur présentant le résumé : Alain JUNG

Résumé : Scientific background: Distant metastasis after treatment is observed in about 20% of Head and Neck Squamous Cell Carcinoma (HNSCC). In the absence of any validated robust biomarker, patients at higher risk for metastasis cannot be provided with appropriate therapy. In order to identify prognostic HNSCC molecular subgroups and potential biomarkers, we have performed genome-wide integrated analysis of four "omic" sets of data.
Material / Patients and Methods: Using state-of-the art technologies, a core of 45metastasizing and 52 non-metastasizing HNSCC patient samples were analyzed at four different levels: gene expression (transcriptome), DNA methylation (methylome), DNA copy number (genome) and miRNA expression (miRNome). Molecular subgroups were identified by a model-based unsupervised clustering analysis, and their clinical relevance was evaluated by survival analysis.
Results: Transcriptome, methylome and miRNome patient subgroups with shorter metastasis-free survival were identified. A contingency analysis uncovered a R1 group of common tumors, which predicts metastasis occurrence with a higher statistical power than individual "omic" data sets. R1 and non-R1 samples display similar DNA copy number landscapes, but more frequent chromosomal aberrations are observed in the R1cluster (especially loss at 13q14.2-3). R1 tumors are characterized by alterations of signaling pathways involved in cell-cell adhesion, EMT, immune response and apoptosis.
Conclusion: Integration of data across several "omic" profiles leads to better selection of patients at risk, identification of relevant molecular pathways of metastasis, and potential to discover biomarkers and drug targets.

INTELLIGENT INTEGRATIVE KNOWLEDGE BASES: BRIDGING GENOMICS, SYSTEMS BIOLOGY AND PERSONALIZED MEDICINE

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Auteur présentant le résumé : Hoan NGUYEN

Résumé : Precision medicine relies on efficient genome annotation, 'omic' data integration and system level analyses to develop new approaches for personalized health care in which patients are treated based on their individual characteristics. We present a knowledgebase (http://decrypthon.igbmc.fr/sm2ph/cgi-bin/home), called SM2PH-Central (from Structural Mutation to Pathology Phenotypes in Human) [1], [2], which is part of an overall strategy aimed at the development of a transversal system to better understand and describe the networks of causality linking a particular phenotype, and one or various genes or networks. It incorporates tools and data related to all human genes, including their evolution, tissue expressions, genomic features and associated phenotypes, in a single infrastructure. It also provides access to systematic annotation tools, including sequence database searches, multiple alignment and 3D model exploitation, physico-chemical, functional, structural and evolutionary characterizations of variants. All information is accessible via standardized reports (gene profiles, error reports, etc.), as well as automated services for specific applications, such as gene prioritization (Gepetto framework). The structuration of the data and information in the SM2PH-Central facilitates the application of intelligent modules to search for hidden patterns and extract pertinent knowledge. For instance, we can cite MSV3d[2], devoted to the characterization of all missense variants and the KD4v system[3], which uses the 3D structures and information to characterize and predict the phenotypic effect of a mutation. A crucial feature of this infrastructure is the ability to create specialized knowledgebase, called SM2PH-Instances, which can be managed independently and allow the integration, management and distribution of domain-specific data (e.g. individual genomes/exomes). An Instance facilitates the construction of test sets to develop, compare and optimize methods to identify the genes and processes involved in the disease. The potential of this infrastructure has been demonstrated in a number of recent studies devoted to complete congenital stationary night blindness[4, 5]).

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TRP CHANNELS: PROGNOSTIC MARKERS AND THERAPEUTIC TARGETS FOR BREAST CANCER ?

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Auteur présentant le résumé : Isabelle DHENNIN-DUTHILLE

Résumé : Scientific background: Breast cancer (BC) is the most frequently occurring cancer in women and has the highest rate of mortality. Evidence is accumulating for the role of ion channels in the development of cancer. Most studied ion channels in BC are K+ channels, which are involved in cell proliferation, cell cycle progression and cell migration, and Na+ channels, which correlate with invasiveness. Emerging studies demonstrated the role of Ca2+ signaling in cancer cell proliferation, survival and migration. Recent findings demonstrated that the expression and/or activity of the transient receptor potential (TRP) channels are altered in several cancers. Among the TRP families, TRPC (canonical or classical), TRPM (melastatin) and TRPV (vanilloid) are related to malignant growth and cancer progression. The expression of TRP channels has also been proposed as a tool for diagnosis, prognosis and/or therapeutic issues of several diseases. In cancer, TRPV6 and TRPM8 have been proposed as tumor progression markers of prostate cancer outcome and TRPC6 as a novel therapeutic target for esophageal carcinoma. Interestingly high levels of TRPC3 expression correlate with a favorable prognosis in patients with lung adenocarcinoma. Although these channels are frequently and abundantly expressed in many tumors, their specific expression, activity and roles in BC are still poorly understood.
Material and methods: TRP channels expression was analysed on human breast invasive ductal adenocarcinoma (IDC, n=60) using immunohistochemistry and real-time PCR. The functional role of TRP channels during BC cell growth and migration was investigated using electrophysiological and molecular biology approaches in primary human breast epithelial cells, and MCF-7 and MDA-MB-231 cell lines.
Results and conclusions: TRPC1, TRPC6, TRPM7, TRPM8 and TRPV6 channels are overexpressed in BC, and the expression profiles of TRPM7, TRPM8, and TRPV6 are associated with pathologic parameters, suggesting their use as prognostic markers. Despite the high frequency of overexpression of TRPC6 observed in IDC samples (78%), no correlation was found between its expression and tumor stage, histological type, or lymph node metastasis. TRPM8 may be considered a good prognostic marker for non invasive well-differentiated ER+ tumors. The expression profile of TRPM7 depends on both invasive and hormonal status: in non invasive ER+ cells, TRPM7 could be proposed as a proliferative marker of poorly differentiated tumors by regulating cell proliferation through calcium influx, whereas these same channel may be proposed as a marker of poor prognosis in aggressive ER– cancers by regulating cell migration through an interaction with cytoskeleton proteins. Finally, TRPV6 expression is highly associated with invasive ER+ tumors, and this channel regulates both migration and invasion, probably through a Ca2+-dependent mechanism, allowing us to propose TRPV6 channel as a marker of poor prognosis.

THE RADIOSENSITIZATION EFFECT OF TITANATE NANOTUBES AS A NEW TOOL IN RADIATION THERAPY FOR GLIOBLASTOMA: A PROOF-OF-CONCEPT

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Auteur présentant le résumé : Céline MIRJOLET

Résumé : Scientific background : Glioblastoma (GBM) is the most frequent primary brain tumor in adults. The median survival time with conventional therapy for patients with multiform glioblastoma is 10 to 12 months. Improving survival in this disease is long overdue. In an attempt to enhance efficacy, it is necessary to radiosensitize or radiopotentiate tumor cells. To decrease the side effects of the systemic drugs, delivering them directly to the tumor using specific nanocarriers is a promising approach. The interest of titanate nanotubes (TiONts) associated with radiotherapy was evaluated in two human glioblastoma cell lines (SNB-19 and U87MG).
Material and methods : Titanate nanotubes were synthetized by the hydrothermal treatment of titanium dioxide powder in a strongly basic NaOH solution. The cytotoxicity of TiONts was evaluated on SNB-19 and U87MG cell lines by cell proliferation assay. The internalization of TiONts was studied using Transmission Electron Microscopy (TEM). Finally, the effect of TiONts on cell radiosensitivity was evaluated using clonogenic assay. Cell cycle distribution was evaluated by flow cytometry after DNA labeling. DNA double-strand breaks were evaluated using ·H2AX labeling followed by fluorescent microscopy analysis.
Results : Cells internalized TiONts through the possible combination of endocytosis and diffusion with no cytotoxicity. Clonogenic assays showed that cell lines incubated with TiONts were radiosensitized with a decrease in the SF2 parameter for both SNB-19 and U87MG cells. TiONts decreased DNA repair efficiency after irradiation and amplified G2/M cell-cycle arrest, in which they are known to be more radiosensitive.
Conclusion : Our results indicated that further development of TiONts might provide a new useful tool for research and clinical therapy in the field of oncology. In a more global project currently in progress, these TiONts are being developed as new nanocarriers. Thus, in a future study, Temodal® will be grafted onto the surface of the nanotubes to improve the effectiveness of radiotherapy by increasing concentration of radiosensitizers into the cells. Moreover, 111Indium labelling have already been grafted to study the distribution / bioavailability of these second generation TiONts by SPECT imaging. Functionalized PEGs have also been covalently coupled to TiONts conjugates to increase TiONts dispersion, stealth and biocompatibility.

PERFORMANCE COMPARISON OF TWO TYPES OF DRUG LOADED IMPLANTS IN A LIVER TUMOR MODEL

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Auteur présentant le résumé : Hadrien D'INCA

Résumé : Drug eluting bead (DEB) is a novel system to treat unresectable HCC [Lammer 2010, Ronnie 2007, Malagari 2010]. DEB consists of polymeric microspheres available in different size ranges and loadable with doxorubicin (DOX) at the dose chosen by the clinician [Lewis 2006]. They have a dual role: act as vascular occlusive agent of tumor feeding arteries and as a local drug delivery system. In our study, we compared two different DEB for bead repartition, tumor damage, drug release from the bead and drug tissue distribution in a preclinical model of liver tumor.
A total of 22 rabbits with liver implanted VX2 tumor were randomly embolized with a fixed volume of DEB (2mg DOX /mg bead). After 3 days, tissue samples were taken in the tumoral and peritumoral tissue, embedded in medium for cryosection and snap-frozen. Sections of 10µm thickness were cut with a cryomicrotome and deposited on a calcium fluoride slide suitable for infrared analysis. The intratumoral/extratumoral location of the occluded vessels and the percentage of necrotized tumor were assessed on HES-stained tissue sections. Drug quantification inside the beads was performed by infrared microspectroscopy. Drug tissue quantification was performed with a fluorescence microspectrometer on the same section as the one used for drug quantification in the beads.
There was no significant difference for the diameter of the two types of DEB (median DEB1: 145µm, median DEB2: 135µm, p=0.66).The majority of beads were located outside the tumor (85%) without significant difference between the two types of beads. The mean concentration of DOX in DEB1 was 0.89±0.68mg/mg beads, corresponding to an elution rate of 55.3%. For DEB2, the signal of DOX could not be detected on any infrared spectrum of any bead, suggesting an elution rate >94% after 3 days. The concentration of DOX in tissue decreased with the distance to the bead but higher drug levels and distance of penetration in tissue were observed for DEB 2 (DEB1 versus DEB2 at 120µm, 1.45 vs 2.02µmol/l, p=0.59 / at 520µm, 0.37 vs 1.20µmol/l, p=0.04 / at 1000µm, <0.29 vs 0.96µmol/l, p=0.007). The percentage of necrotized tumor was superior to 99% of the tumor surface, not significantly different between the products (p=0.52). The mean percentage of necrotized liver parenchyma surrounding the tumor was not significantly different between the products (DEB1 versus DEB2: 35% vs 48%, p=0.47).
To conclude, the two drug eluting beads tested showed different release properties but an equivalent efficacy on tumor necrosis. Supplementary studies are needed to determine if a reduction of drug concentration loaded in beads is possible while maintaining a good level of tumor cytotoxicity.

PHARMACOLOGICAL INHIBITION OF THE EGFR/MTOR/HIF-1 SIGNALING PATHWAY SENSITIZES HEAD AND NECK SQUAMOUS CELL CARCINOMA (HNSCC) CELL LINES TO IONIZING RADIATIONS.

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Auteur présentant le résumé : Pierre COLIAT

Résumé : Backround: Therapeutic management of HNSCC are mainly based on surgery and radio- or chemoradiotherapies. In addition, targeted therapy agents raised against the Epidermal Growth Factor Receptor (EGFR) can be used in addition to ionizing radiations for treatment of locally advanced metastatic lesions. However, the overall survival is poor, with less than 50% of the patients being alive 5 years after treatment. HNSCC resistance to treatment may involve tumor hypoxia. Indeed, it has been shown that the stabilization of Hypoxia Inducible Factors (HIF), that are key regulators of the cell adaptation to hypoxia, correlates with tumor resistance to ionizing radiations and adverse prognosis.
Pharmacological approaches using targeted agent therapies that inhibit the EGFR/mTOR pathway with specific inhibitors have shown efficacy in some solid tumors such as NSLCC (Non Small Lung Cells Cancer). Moreover, some studies suggest that the use of mTOR inhibitors sensitizes radioresistant cells and anti-EGFR resistant cells.
Method: Pharmacological treatment of HNSCC cell lines (SQ20B and CAL27) was performed by using Rapamycin (mTOR inhibitor) and Cetuximab (anti-EGFR antibody), with or without radiotherapy. The effect of these treatments on cell survival was measured with proliferation assays and clonogenic survival assays. The molecular impact of these treatments on HIF-1 inhibition and on PI3K/AKT and MAPK pathways activation was assessed by probing cell culture protein extracts with a western blot approach.
Results: A reduction of more than 50% of the cell survival fraction, as measured with clonogenic assays, was observed upon a treatment combining ionizing radiations and low doses of both Rapamycin and Cetuximab, and correlated with efficient inhibition of HIF-1 alpha protein accumulation in tumor cells. Interestingly, the use of chemotherapy alone did not reduce the cell survival fraction. The analysis of the activation of the oncogenic signaling pathway showed that ionizing radiations induce the phosphorylation of the ERK MAPK. This activation is no longer observed when cells are cotreated with both chemo- and radiation therapy.
Discussion: These preliminary results suggest: i) an increased efficacy of dual targeting of the EGFR / mTOR signaling pathway by a combination of Rapamycin and Cetuximab ; ii) a potential role of this treatment in increasing the cell sensitivity to radiotherapy . The efficacy of this drug combination in radiosensitizing tumor cells is currently under investigation with in vivo approaches using xenografts in nude mice models.

THROMBOSPONDIN-BINDING TAX2 PEPTIDES AS NEW EXCITING MOLECULES FOR CANCER TREATMENT

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Auteur présentant le résumé : Albin JEANNE

Résumé : Background: Thrombospondin-1 (TSP1) is a ubiquitously expressed multimodular glycoprotein implicated in many pathophysiological processes including cell adhesion, proliferation, apoptosis, inflammation and cardiovascular responses. These biological functions modulated by TSP1 make it a key actor in tumor microenvironment and a potent target for the development of useful therapeutic tools against tumorigenesis and metastasis. As the anti-angiogenic properties of TSP1 are mediated by its C-terminal domain interacting with the CD47 membrane receptor, we try to identify peptides acting as specific antagonists for TSP1:CD47 interaction.
Methods: Peptides were designed using in silico normal mode analysis (CHARMM) and docking experiments between TSP1, CD47 and CD47-derived peptides were conducted (GRAMM-X, RosettaDock, Autodock). Molecular interactions were assessed by co-IP and ELISA binding assays. The consequences of peptide treatment regarding angiogenesis were investigated using in vitro (endothelial cell migration, invasion and tube formation) and ex vivo (mouse aortic ring assay) models. We then investigated in vivo the therapeutic potential of such peptides considering two models of melanoma in C57bl/6 mice, a subcutaneous B16F1 allograft model and an experimental model of B16F10 lung metastasis, using follow-up micro-computed tomography (µCT) studies correlated with MRI and histopathological analysis.
Results: Molecular dynamics studies led to identify two CD47-derived peptides (TAX2l and its disulfide-bound analogue TAX2c) able to bind TSP1 specifically and to antagonize TSP1:CD47 interaction, as confirmed by co-IP and ELISA binding assays. Interestingly, these peptides inhibit angiogenesis both in vitro and ex vivo, while TSP1 (C-ter) interaction with CD47 is known to inhibit angiogenesis. Indeed, the proposed peptides induce in an original manner a molecular switch of TSP1 from CD47 to CD36 binding, responsible for a VEGF/VEGFR2-induced angiogenesis inhibition. Moreover, TAX2c induces a strong tumor necrosis and substantially disturbed tumor vascularization in a subcutaneous B16F1 tumor model, but also inhibits the dissemination and growth of experimental B16F10 lung metastases.
Conclusion: Consistent with in vitro and ex vivo observations, longitudinal µCT monitoring in two murine models of melanoma highlighted strong anti-tumor, anti-angiogenic and anti-metastatic properties of the in silico-designed TAX2c peptide, which could therefore represent new exciting molecules for cancer treatment. Further studies will focus on investigating the anti-tumor efficiency of such a peptide considering a wider range of preclinical solid tumor models.

VENDREDI 28 JUIN 2013

SESSION 3 :

VIRUS ET CANCER

CONFERENCES PLENIERES

COMMUNICATIONS ORALES SELECTIONNEES :

SESSION 3

SPECIFIC METHYLATION OF HPV16 E2BS#1 AND #2 IN CERVICAL CANCER SAMPLES DETERMINED WITH HRM PCR METHOD

Auteurs et adresses : Elise Jacquin(1), Baraquin Alice(2), Rajeev Ramanah(2), Xavier Carcopino(3), Adrien Morel(1), Séverine Degano-Valmary(1,2), Ignacio Bravo(4), Silvia de Sanjose(4), Didier Riethmuller(1,2), Christiane Mougin(1,2), Jean-Luc Prétet(1,2)
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(4)Catalan Institute of Oncology (ICO), Barcelona, Spain

Auteur présentant le résumé : Elise JACQUIN

Résumé : Scientific background
A persistent infection with high-risk HPV (HR-HPV) is the main risk factor for progression of cervical lesions towards cancer. The introduction of molecular biology in cervical cancer screening has improved the sensitivity of tests for the detection of precancerous lesions. However, the specificity of the screening tests based on the detection of HR-HPV DNA is limited. Identification of new biomarkers permitting to discriminate women with the highest risk of lesion progression remains a challenge and involves the comprehension of molecular mechanisms of HPV16-associated carcinogenesis.
Material / Patients and Methods
A new methylation specific high resolution melting (HRM) PCR targeting the two main E2 binding sites (E2BS#1 and #2) and Sp1 binding site involved in HPV16 oncogene regulation was developed and validated. The technique was applied to 119 HPV16+ cervical smears with different cytological diagnosis to assess the diagnostic value of E2BS methylation.
Results
The HRM PCR was validated in terms of specificity, intra- and inter-assay reproducibility. Standards were developed to permit a quantitative assessment of E2BS#1, E2BS#2 and Sp1 binding site methylation level. The methylation frequency of the target CpGs was 47% in cancer samples whereas most if not all samples diagnosed as normal, low and high grade lesions harbored unmethylated HPV16 CpGs. However, the level of methylation of the target CpGs varied from 10 to 60%. Results obtained with HRM PCR were then compared to those obtained with pyrosequencing as a confirmation test. A perfect agreement was obtained between the two techniques. In a clinical point of view, our HRM PCR method allows the identification of women with a cervical cancer with a specificity of 97% and a sensitivity of 44%
Conclusion
We demonstrated that HRM PCR is a specific, highly reproducible and reliable single-tube assay that allows a quantitative assessment of HPV16 E2BS methylation from cervical smears that harbor multiple copies of HPV16 DNA as reflected by the viral load. The prognostic value of the E2BS#1, E2BS#2 and Sp1 binding site methylation deserves to be investigated alone or in association with other markers such as load, integration or transcript levels. The HRM PCR offers great opportunities to explore HPV16 methylation in other HPV-related cancers such as head and neck cancers that remain a major public health burden.

DEVELOPMENT AND VALIDATION OF A REAL-TIME PCR ASSAY USING COBAS® FOR THE DETECTION AND GENOTYPING OF HUMAN PAPILLOMAVIRUS IN CERVICAL OR BUCCAL SMEARS AND IN FORMALIN-FIXED EMBEDDED HEAD AND NECK TUMORS

Auteurs et adresses : Alexandre Harlé 1,2,3 ; Julie Guillet 2,3,4, ; Marie Rouyer 1 ; Jean-Louis Merlin1,2,3
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Auteur présentant le résumé : Alexandre HARLE

Résumé : Scientific background: Human Papillomavirus (HPV) is the most common sexually transmitted infections in developed countries. HPV has recently become one of the leading cause of cervical and oral cancers. The current HPV assays for the detection in formalin-fixed paraffin embedded tumors (FFPET) are often time consuming and expensive. Some real-time PCR assays are commercially available in virology field for the detection of HPV DNA in cervical smears but no real-time PCR assay has been validated for the detection of HPV DNA in FFPET and for molecular biology purpose. We described here the validation of a new method based on commercial kits adapted from virology able to detect HPV DNA routinely usable in cervical or buccal smears as well as in FFPET using real-time PCR.
Material / Patients & Methods: 32 samples from 32 patients have been analyzed for this method development assay. DNA was extracted using Roche extraction DNA kit according to the manufacturer protocol. Samples have been assessed using Roche Cobas® z480 and Cobas® HPV kits have been adapted to allow the detection of HPV DNA in FFPET as well as in cervical or buccal smears samples. All results obtained with the Cobas® assay were compared with those achieved using our standard PCR assay with electrophoretic detection of HPV DNA used routinely. This assay is designed to specifically detect DNA from HPV 16, 18, 33 and concurrently DNA from HPV 6, 11, 13, 15, 16, 18, 30, 31, 32, 33, 34, 35, 39, 40, 42, 45, 51, 52, 53, 56, 58, 61, 66, 80. Cobas® HPV assay can specifically detect DNA from HPV 16 and 18 and concurrently DNA from high risk HPV 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66 and 68. Fleiss kappa statistics have been used to compare both methods.
Results: Among the 32 samples, 13 were from FFPET and 19 from cervical or buccal smears. Four samples were non interpretable, 6 were positive for HPV16 DNA, 3 for both HPV 16 and 33 DNA, 1 for HPV18 DNA, 1 for HPV33 DNA, 1 for HPV6 or 11 DNA, 1 for both HPV 6 or 11 and consensus high risk HPV DNA and 15 were negative. Among the 32 samples, the results achieved with both techniques were found identical in 31/32 cases ($k=0.955$; $p<1.10\text{-}9$). Only one discordance was found in one sample which was positive for HPV 6 or 11 DNA using our standard PCR assay, which is not included in the Cobas® assay.
Conclusion: This new real-time PCR Cobas® assay allows to detect accurately and rapidly (<3 h) high risk HPV DNA in cervical or buccal smears as well as in FFPET samples. The results are statistically comparable with our standard PCR assay. Cobas® assay can now be used routinely, warranting shorter delay for reporting of the results and lower human resources-related costs of the analyses.

SESSION 4 :

ECOSYSTEME TUMORAL

CONFÉRENCES PLÉNIÈRES

INTEGRINS AS THERAPEUTIC TARGETS IN ONCOLOGY

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Auteur présentant le résumé : Dontenwill Monique

Résumé : Integrins are cell surface heterodimeric receptors primarily involved in integrating the extracellular environment to the intracellular environment thus defining specific cell biological answers. Due to their implications in various biological processes, the complexity and diversity of integrin functions and regulation has attracted much interest in the scientific world. As crucial players in cell attachment, movement, growth, survival and differentiation, their roles in cancer have been suspected and confirmed these last years.

The integrin family contains about 24 different types of αβ heterodimers. The 24 known integrin heterodimers can be classified as RGD-binding integrins, α4 integrins, leucocyte adhesion integrins, laminin-binding and collagen-binding integrins. Many different heterodimers can be expressed on a single cell and each can trigger multiple intracellular signalling cascades. Depending on the cell/tissue context, the biological effect of an integrin can vary dramatically. At present the crucial pharmacological issue is to determine which particular integrin in which specific context may be a true target. Currently, preclinical and clinical studies mainly point to the implication of RGD-binding integrins as important players in tumor progression, aggressiveness and recurrence.

The first highlighted role of integrins in cancer concerned tumoral angiogenesis. High level of αvβ3/β5 integrin has been found in activated endothelial cells participating in tumor angiogenesis compared to quiescent endothelial cells. Inhibition of their interaction with their ligands has thus been targeted for blocking endothelial cell-mediated angiogenesis associated with tumor growth. Cilengitide, the first integrin inhibitor reaching the clinic, has been used this way in the treatment of different cancers in phase II trials and hence in a phase III trial for glioblastoma. Beside αvβ3/β5 integrin, α5β1 integrin moved recently as a therapeutic target to block tumoral neoangiogenesis. Integrins are also directly involved in tumoral cell behaviour participating in their capability to proliferate, to move from the primary tumor towards metastatic niches and to resist to radio-chemo-therapies. More recently, integrins were also established as new actors in cancer stem cell biology.

Integrin antagonists may therefore inhibit tumor progression by blocking crucial events in both the tumor microenvironment and the tumor cells themselves. Anti-integrin drugs that are being currently in process of development are designed to specifically block the interaction of integrins with their ligands. They can be classified into three major categories: 1) monoclonal antibodies, 2) peptide inhibitors and 3) non peptidic small molecule inhibitors. They are mainly pre-clinically investigated and few are undergoing different phases of clinical trials.

Current knowledge on integrins in the field of oncology will be presented, associating both preclinical and clinical data with consideration of concerns that are associated with direct targeting of integrins.

LES ADIPOCYTES PERI-TUMORAUX : DE NOUVEAUX ACTEURS DE LA PROGRESSION TUMORALE MAMMAIRE

Auteurs et adresses : Pr Catherine MULLER-STAUMONT
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Auteur présentant le résumé : Pr Catherine MULLER-STAUMONT

Résumé : Parmi les cellules qui constituent le stroma tumoral du cancer du sein, les adipocytes sont probablement celles donc le rôle a été le moins bien caractérisé malgré le fait que les adipocytes représentent un des types cellulaires prédominants de ce stroma. L'identification d'un rôle des adipocytes dans la progression tumorale est un point important en médecine humaine puisqu'il a été montré que l'obésité est un facteur pronostique négatif dans le cancer du sein où elle est associée à une augmentation des métastases et à une diminution de la survie globale Dans le cancer du sein, nous avons montré (y compris dans les tumeurs mammaires humaines) que les adipocytes au front invasif de la tumeur présentent des modifications spécifiques marquées par une délipidation, une diminution des marqueurs adipocytaires et une surexpression de cytokines pro-inflammatoires (IL-6, IL-1β) et de protéases (dont la MMP-11). Ces cellules, que nous avons nommé des Cancer Associated Adipocytes (ou CAAs) stimulent *in vitro* et *in vivo* les capacités invasives des cellules tumorales mammaires (Dirat *et al*, Cancer Research :71:2455-2465, 2011). Ces résultats montrent donc qu'un dialogue bidirectionnel s'établit entre cellules tumorales et CAAs permettant de renforcer la progression tumorale. De façon très intéressante, des résultats plus récents suggèrent que ces CAAs pourraient être retrouvés au front invasif de nombreuses tumeurs tels que le cancer du colon, de la prostate et de l'ovaire disséminé. Outre l'apparition des CAAs, nos derniers résultats montrent que les cellules tumorales vont être capables de « réorienter » les adipocytes jusqu'à un phénotype fibroblastique. Ces fibroblastes dérivés des adipocytes représentent une sous population de CAFs (pour Cancer-Associated Fibroblats) et participent à la réaction desmoplastique dans le cancer du sein (Bochet et al, Cancer Research, sous presse). Enfin, le dialogue entre tumeur et tissu adipeux fait aussi appel à un échange d'acides gras libres (AGLs) entre ces deux compartiments, AGLs qui vont être utilisés pour favoriser le métabolisme de la tumeur. Nous avons donc montré que le tissu adipeux péri-tumoral présente des modifications spécifiques telles que l'inflammation, l'augmentation de la réaction fibrotique, la libération d'AGLs qui toutes concourent à favoriser la progression tumorale. De façon intéressante, les modifications induites dans le tissu adipeux à proximité des tumeurs présentent de grandes similitudes avec celles observées au cours de l'obésité. Ainsi, notre hypothèse est que les adipocytes présents à proximité des tumeurs seraient, chez l'obèse, encore plus enclins à stimuler la dissémination locale et à distance ce qui pourrait expliquer le pronostic défavorable observé chez ces patientes, hypothèse sur laquelle nous travaillons actuellement.

SESSION 5 :

IMMUNOTHERAPIE

CONFERENCES PLENIERES

CD160 : UNE NOUVELLE CIBLE THERAPEUTIQUE ANTI-ANGIOGENIQUE

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Auteur présentant le résumé : Philippe Le Bouteiller

Résumé : Dans le cadre d'une identification de récepteurs spécifiques des lymphocytes de l'immunité innée nous avons décrit CD160. Il s'est avéré par la suite que ce récepteur est aussi exprimé à la surface des cellules T et des cellules endothéliales après activation. Nous avons montré que l'engagement spécifique de ce récepteur (dont le gène est conservé chez l'homme et plusieurs espèces de mammifères) par un anticorps monoclonal agoniste, CL1-R2, induit l'apoptose des cellules endothéliales activées. Des études *in situ* on clairement révélé que CD160 est exprimé par des cellules endothéliales du cancer du colon. Le traitement de souris transplantées avec des tumeurs très vascularisées de type mélanome B16 ou fibrosarcome, combiné à une chimiothérapie, entraîne une diminution de la densité vasculaire tumorale. L'anticorps CL1-R2 normalise la structure des vaisseaux tumoraux restants qui deviennent matures et fonctionnels (lumière ouverte, entourés de péricytes, mieux perfusés). Ces effets entraînent une réduction significative de la croissance tumorale et du taux de survie des souris traitées par CL1-R2 et chimiothérapie par rapport à des souris traitées uniquement par chimiothérapie. Ces études *in vivo* démontrent que le récepteur CD160 est une nouvelle cible thérapeutique visant des cancers hautement vascularisés, en association avec une chimiothérapie.

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Fons P, Chabot S, Cartwright JE, Lenfant F, L'Faqihi F, Giustiniani J, Herault JP, Gueguen G, Bono F, Savi P, Aguerre-Girr M, Fournel S, Malecaze F, Bensussan A, Plouët J, & Le Bouteiller P. (2006) Soluble HLA-G1 inhibits angiogenesis through apoptotic pathway and by direct binding to CD160 receptor expressed by endothelial cells. Blood, 108, 2608-25

Le Bouteiller P, Tabiasco J, Giustiniani J & Bensussan A. (2012) Nouvelle thérapie anti-angiogénique indépendante du VEGF et ciblant le récepteur CD160. *Médecine Sciences*, 28 : 37-38

CYTOTOXICITE DES CELLULES NK DANS LES HEMOPATHIES MALIGNES

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Auteur présentant le résumé : Nicolas Dulphy

Résumé : Les cellules Natural Killer (NK) font l'objet, depuis quelques années, d'un intérêt croissant du fait de leur capacité à éliminer les cellules tumorales. La régulation particulière des fonctions NK, indépendante d'un antigène, pourrait permettre l'utilisation de ces cellules effectrices comme outil d'immuno-thérapie adoptive.
L'étude des sous-populations NK chez des patients au diagnostic de Lymphome Diffus à Grandes Cellules B (DLBCL) ou de Leucémie Aigue Myéloblastique (LAM) révèle des défauts de la fonction cytotoxique des
cellules NK face aux cellules tumorales.
- Les patients DLBCL, habituellement traités par la combinaison d'une chimiothérapie et d'un anticorps monoclonal dirigé contre le CD20 exprimé sur les cellules lymphomateuses, présentent des déficits des
cellules NK dans l'expression du récepteur au domaine Fc des Ig de type III (CD16) et dans la cytotoxicité cellulaire associée aux anticorps (ADCC) (Danielou-Lazareth A. *et al*. Eur J Immunol. 2013. **43**:1383-8).
- Certains patients LAM présentent des défauts phénotypiques et fonctionnels NK associés à un risque accru de rechute. L'étude de l'interaction entre la cellule leucémique et la cellule NK suggère un rôle
actif des cellules tumorales dans la suppression des fonctions NK, d'une part en réduisant leur sensibilité à la cytotoxicité mais aussi en inhibant l'expression des récepteurs activateurs à la surface de la cellule effectrice (Khaznadar Z. *et al*. Manuscrit en préparation).
Ainsi, la réduction de la cytotoxicité des cellules NK observée dans ces deux exemples suggère l'importance d'une inhibition des cellules NK pour assurer le développement des hémopathies malignes. Des stratégies pour rétablir ces fonctions cytotoxiques ou un usage ciblé des cellules NK dans des immunothérapies personnalisées pourraient donc constituer une avancée significative pour le traitement des patients.

LES INHIBITEURS DE BRAF DANS LES MELANOMES

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Auteur présentant le résumé : Nicolas Dumaz

Résumé : Le mélanome est un cancer cutané qui se développe à partir des mélanocytes, cellules de l'épiderme dont la fonction est de synthétiser la mélanine qui protège la peau des effets délétères des ultraviolets. L'incidence du mélanome en Europe, qui est d'environ 10 cas pour 100 000/an, est en constante augmentation malgré les campagnes de prévention et de dépistage. Ainsi le mélanome est devenu le cancer le plus fréquent chez les jeunes adultes et une des plus fréquentes causes de décès par cancer dans la population active. En effet, alors que le mélanome ne représente que 10% des tumeurs malignes cutanées, il est la cause de 90% des décès par cancer de la peau car c'est une tumeur très agressive et qui est résistante à la chimiothérapie et la radiothérapie. Les thérapies ciblées du mélanome, qui émergent depuis peu, apportent de grands espoirs dans la prise en charge du mélanome métastatique et devraient progressivement changer la façon dont le mélanome est traité. Ces nouvelles thérapies ciblent les voies de transduction du signal qui sont impliquées dans le développement du mélanome et en particulier la voie MAPK (Mitogen Activated Protein Kinase) qui joue un rôle majeur dans la genèse du mélanome. La voie MAPK est activée en aval de Récepteurs à activité Tyrosine Kinase (RTK), lorsqu'ils sont stimulés par leurs ligands. Les RTK activent indirectement la petite GTPase RAS qui recrute les sérine/thréonine kinases RAF. Les kinases RAF, dont il existe trois isoformes (ARAF, BRAF et CRAF) vont stimuler une cascade de phosphorylations impliquant les kinases MEK et ERK. ERK phosphoryle de nombreux effecteurs qui régulent l'expression des gènes impliqués dans la prolifération, la différenciation et la survie cellulaire. La mutation la plus fréquente dans les mélanomes cutanés est la mutation V600E de la kinase BRAF. Cette mutation, induit une activation constitutive de la kinase BRAF et ainsi de la voie MAPK. Elle est retrouvée dans 50% des mélanomes à extension superficielle et moins fréquemment dans les autres sous types de mélanome. Depuis Février 2012, un inhibiteur de $BRAF^{V600E}$ (vemurafenib) dispose de l'AMM (Autorisation de Mise sur le Marché) pour le traitement des mélanomes contenant une mutation de BRAF. Cet inhibiteur présente un taux de réponse de 50%, ce qui est exceptionnel par rapport aux autres thérapeutiques disponibles. Malheureusement des résistances au traitement apparaissent fréquemment après quelques mois entraînant des rechutes. Les études actuelles visent à comprendre les mécanismes moléculaires de résistance dans le but de les traiter et éventuellement de les prévenir.

COMMUNICATIONS ORALES SELECTIONNEES :

SESSIONS 4 ET 5

EPIGENETIC CONTROL OF TUMOR CELL INVASION

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Auteur présentant le résumé : Mathilde POPLINEAU

Résumé : Invasive properties of cancer cells require critical changes in gene expression. Proteases must be expressed for the degradation of the extracellular matrix (ECM), the proteolytic activation of matrix proteins and the release of bioactive molecules such as growth factors, cytokines, receptors and adhesion molecules. Among these proteases, the matrix metalloproteinase (MMP) family members play a crucial role in the ECM breakdown and remodeling of tissues during tumor invasion. The introduction of epigenetic strategies in the therapeutic arsenal against cancer led to the need to evaluate the effects of such therapeutic approaches on cell behavior. Here we focused our attention on the effects of epigenetic modulators, a DNA hypomethylating agent and histone deacetylase inhibitors (HDAC inhibitors or HDI), on the expressions of MMP-1,-2, and -9 in the human HT1080 fibrosarcoma cell line. First, we showed that the hypomethylating drug 5-aza-2'deoxycytidine (5-azadC) increases MMP-1, -2, -9 expressions both at the mRNA and protein levels. These changes in gene expression are associated with (i) a global DNA demethylation and with (ii) modifications in chromatin supra-organization which globally correspond to a more decondensed chromatin. Moreover, 5-azadC is able to increase the invasive properties capability of the HT1080 cells mainly via MMP-1 transcription-dependent expression. This enhancement of transcription occurs through (i) Sp1 recruitment, (ii) chromatin remodeling and (iii) in absence of full demethylation on the MMP-1 gene promoter. Using different HDIs reveals that HDACs could potentially play a role in MMP-1 expression. The pan-HDI trichostatin A (TSA) act in synergy with 5-azadC and is able to modulate MMP-1 expression and nuclear texture, but only after DNA demethylation. In contrast, the HDAC class I inhibitor, MS-275, which display additive effect with 5-azadC, is able to induce, alone, MMP-1 gene expression through chromatin remodeling and p300 recruitment to its promoter. These data suggest that epigenetic mechanisms play a crucial role in MMP-1 expression control in HT1080 cells thus influencing the invasive potential of these cells.

EVALUATION OF NON PEPTIDIC ANTAGONISTS OF RGD-INTEGRINS.

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Auteur présentant le résumé : Anne-Marie RAY

Résumé : Scientific Background
Integrins are cell adhesion receptors implicated in a variety of tumoral processes such as angiogenesis, migration, invasion or survival. They emerge as promising anticancer targets. Numerous studies aim to characterize efficient specific antagonists of integrins. Affinity and selectivity of integrin antagonists are nowadays mainly extrapolated from adhesion assays on purified integrins. Investigations about their biological effects in cells expressing different integrins reveal more complex mechanisms. In this work we compared the efficacy of different RGD-like integrin antagonists on glioma cell adhesion and migration.
Material and Methods
Two cell lines, U87MG and U373, expressing both α5β1 and αvβ3 integrins, were used and effects of antagonists evaluated in classical adhesion tests on fibronectin and vitronectin as well as in wound healing and single cell tracking migration assays.
Results
Data show that U87MG cells were highly dependent on α5β1 integrins for adhesion to fibronectin and on αvβ3 integrins for adhesion to vitronectin. Inhibition of single cell migration was obtained with α5β1 integrin antagonists although αvβ3 integrin antagonists rather increased the cell migration. U373 cells were mainly dependent on αvβ3 integrins for adhesion and single cell migration was only increased by antagonists. In wound healing assays, collective cell migration involved α5β1 and αvβ3 integrins for both cell lines and was inhibited by both class of antagonists.
Conclusion
Our results underline the need to clearly define the cell system used to evaluate the effects of integrin antagonists and that single cell migration of U87MG cells overexpressing or depressed for α5 integrin is a pertinent model to characterize antagonist specificities for α5β1 or αvβ3 integrins.

IL-17 A, B AND E CONTRIBUTE TO BREAST CANCER PATHOLOGY WITH GENERATION OF PRO-ONCOGENIC LOW MOLECULAR FORMS OF CYCLIN E

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Résumé : Background
The IL-17 family of cytokines is composed of six members, IL-17A to IL-17F with IL-17A as the prototypic member. This interleukine was described as the signature of the recently T helper 17 (TH17) cell subset (Park H, Nat Immunol 2005; Harrington LE, Nat Immunol 2005). A large body of evidences has revealed important roles for this cytokine and TH17 cells, in the development of allergic and autoimmune diseases as well as in protective mechanisms against bacterial and fungal infections (Ouyang W, Immunity 2008) and have gained prominence in cancer, particularly in breast carcinomas. In mouse models of breast cancers, IL-17A promotes tumour growth and angiogenesis (Du JW, Mol Med Rep. 2012) whereas proliferation and invasiveness were demonstrated with human cell lines (Zhu X, Breast Cancer Res. 2008).
Recently, two other members were found to be present in the breast tumor microenvironment: IL-17B produced by malignant cells and IL-17E (IL-25) by mammary epithelial cells (MECs) (Furuta S, Sci Transl Med. 2011).
While IL-17A is recognized by the IL-17RA/RC complex, the IL-17RB homodimer and the IL-17RA/RB heterodimer serve as receptors for IL-17B and –E respectively but their specific roles are still elusive. Thus, opposite functions were demonstrated when exogenous IL-17E is added to breast cancer cell lines (Furuta S, Sci Transl Med. 2011; Rubio MF, Oncogene 2006) and a pro survival role was suggested for IL-17B (Furuta S, Sci Transl Med.
2011).
Methods
Several breast cancer cell lines were used (MCF-7, T47D) as well as cell line developed in our lab (IJG-1731). RT-PCRs were done to detect mRNA coding for IL-17 receptors. Clonogenic assay and cell signaling studies were performed on all these cell lines.
Results
We confirmed that IL17-A, -B or –E participate to the tumor development increasing the clonogenic activity of breast cancer cells in vitro, promoting the phosphorylation of FAK, c-Raf and the p70S6 kinase, a downstream target of mTOR and an important mediator of mTOR function.
We analyzed the expression of cyclin E, a key regulator of the cell cycle and report, for the first time, that addition of IL-17A, -B or -E induces its cleavage into low molecular weight forms (LMW-cyclin E) which are associated with tumor genesis and poor disease specific survival (Duong MT, Plos Genetics 2012).
Conclusions
This work should demonstrate that IL-17 cytokine family could have an important role to breast cancer pathophysiology, regulating cancer progression and could represent an interesting target for immunotherapy.

HYPOMETHYLATING AGENT 5-AZA CYTIDINE EXPOSURE TO IMPROVE AP1903 TREATMENT FOR APOPTOSIS INDUCTION OF ICASP9/ΔCD19 GENE MODIFIED T CELLS

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Auteur présentant le résumé : Elodie BOLE-RICHARD

Résumé : Haematopoietic transplantation may result in graft versus host disease (GvHD). T-lymphocyte depletion of the bone marrow graft can prevent GvHD, while increasing the risk of rejection and reducing the anti-leukemic effect. An alternative issue is to use a suicide gene system that allows in-vivo conditional T cell depletion. Based on our experience reporting drawbacks of the suicide gene HSV-tk expressing donor T cells, we investigate the use of human derived inducible caspase 9 (iCasp9/iC9) and truncated CD19 (ΔCD19) expressing T-cell. This allows rapid apoptosis after exposure to a Chemical Inducer of Dimerization (CID; AP1903, Bellicum Pharmaceuticals).As reported in-vivo by others, we found a drug responsiveness of some iC9/ΔCD19+ gene modified cells (GMC) after CID in-vitro exposure. Such findings are also described with other suicide gene approaches (HSV-tk/GCV or CD20/Rituximab). The aims of this work were 1/ to better characterize the CID responsiveness and 2/ to evaluate a new means to improve the treatment. We generated, by retroviral transduction (SFG.iCasp9.2A.ΔCD19), iC9/ΔCD19+ Jurkat, CEM and HUT cell lines. Transduction efficiency was 57.7±13.6 (n=3). After induction of apoptosis with CID, GMC were efficiently eliminated. However, we found that 1.47% ± 0.67% (n=5) of ΔCD19+ MACS-selected escaped after CID in-vitro exposure. GMC were serially exposed to increase the doses (0.1nM to 10nM) of CID to generate resistant cell lines (GMCR). Drug response was assessed by 7-AAD/Annexin V staining and surviving GMC were cloned to isolate GMCR. Compared to sensitive GMC (GMCS), we showed that GMCR are lower ΔCD19 expressers (MFI= 30 fold less). While retroviral vector is based on 2A system, allowing stoichiometric iC9/ΔCD19 proteins production, we hypothesize that GMCR are also the lower iC9 expressers. This has been confirmed by western blot (WB) for both full length and cleaved iC9. A hypomethylating agent could increase transgenes expression in order to improve iC9 GMC response. A good candidate could be the 5-aza. To assess this hypothesis, CEM-GMCS and -GMCR were treated 4 days with 5-aza (1µM). We have shown by cytometry that 5-aza exposure of CEM-GMCR increase the ΔCD19 expression (MFI= 5 fold more). Moreover, WB analysis confirms the increase of the iC9 protein expression. As expected, drug response is improved after 5-aza treatment. The percentage of CEM-GMCR mortality induced by CID vs 5-aza+CID was 3.8% vs 62.5% respectively. Stable CD4 expression suggests that transgenes expression has been mainly targeted.In conclusion, we have generated CID resistant T cell lines and determined that low responders are iC9/ΔCD19 low expressers. Moreover, we have shown that 5-aza treatment improves the expression of transgenes and leads to restore the CID killing. This approach can be applied in further clinical trials in order to improve killing of iC9 expressing T cells used in hematopoietic transplantation or to secure cell therapies.

POSTERS

POSTERS SELECTIONNES

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PO3

ERALPHA36, UN MARQUEUR PREDICTIF DE LA REPONSE THERAPEUTIQUE ET DU POTENTIEL METASTATIQUE DES TUMEURS MAMMAIRES ?

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Auteur présentant le résumé : Clémence CHAMARD

Résumé : Breast cancer is the main cause of cancer-induced morbidity and mortality in women. Breast tumors are usually classified according to their ER·66 status. Such a classification led to the use of endocrine therapeutic agents against ER-positive tumors [ER+]. Nevertheless, numerous therapeutic failures are observed due to unclear resistance mechanism.
ER·66 was considered as the unique functional estrogen receptor in hormone sensitive breast tumor until the recent identification of membrane bound new estrogen receptors: the G protein coupled estrogen receptor (GPER) and the 36kDa ER· splice variant (ER·36). Surprisingly, ER·36 stimulates cell proliferation in response to tamoxifen treatment and could therefore be involved in the acquired resistance to this compound. Moreover, a high ER·36 expression level correlates with a short term survival for ER·66-negative patients as well as enhanced tumorigenesis and metastatic potential of triple-negative cells in vitro.
The aim of our project was to improve the classification of so-called "ER-positive" breast tumors by taking into account the key role of ER·36 and GPER in the control of non genomic estrogen response and metastatic potential. We set up a retrospective study, performed on hundreds of breast tumor samples, in order to better define the field of acceptance of [ER+] versus [ER-] classification and to help the clinicians choosing the best therapeutic compounds. We addressed two main questions:
1- Are ER·36 and GPER predictive markers of therapeutic response in ER-positive tamoxifen-treated patients [ER+,TAM+]?
2- Is a high ER·36 or/and GPER expression level of poor prognosis because these receptors stimulate tumor progression and metastatic potential?
ER·36, GPER and metastatic marker expressions were measured by real-time PCR in almost 100 [ER+,TAM] as well as 60 triple-negatives [ER-,TAM-] tumor samples. Then, we performed statistical analyses between gene expression levels and clinical parameters (grade, survival, treatment).
Modeling of the potential relationship between the genes tested using nonlinear correlation analyses, Bayesian inference and transfer entropy computation led to the characterization of complex network connecting non genomic estrogen signaling and metastatic process. Hence, ER·36 expression is strongly related to GPER, Snail1, Vim and MMP9A in [ER+, TAM+] samples. This suggests that, after tamoxifen treatment, ER·36 may stimulate the metastatic potential of [ER+] tumor in vivo. Such a model will be first tested in vitro in hormone-dependent or triple negative cell lines.
Taken together, the results from this project should lead to (i) a better understanding of the breast tumor hormone sensitive status, (ii) a validation of new predictive markers of response in order to improve therapeutic orientation, (iii) the potential discovery of new therapeutic targets in triple-negative tumors.

PO9

ACTIVATION OF P38MAP KINASE IN ESTROGEN POSITIVE INVASIVE BREAST CANCERS : RELATION WITH HER2 AND DOWNSTREAM SIGNALING PHOSPHORYLATED PROTEINS EXPRESSION.

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Auteur présentant le résumé : Jean-Louis MERLIN

Résumé : Background : P38 kinases are members of the mitogen-activated protein kinase (MAPK) family. In breast cancers MAPK, as well as PI3 kinase-AKT pathway signaling proteins have major implication in molecular oncogenesis and are extensively investigated as putative targets for therapy. The present study reports the investigation of the expression of P38MAPK and its phosphorylated form (p-P38MAPK) in clinical specimens of invasive breast carcinomas in relation with estrogen receptor and HER2 expression, as well as MAPK and PI3K signaling phosphorylated proteins. **Methods:** The expression of P38MAPK and p-P38MAPK as well as p-AKT, p-GSK3β, p-S6 kinase, p-MEK1, p-ERK1/2 were semi-quantitatively assessed using multiplex bead immuno-assay. The analyses were performed retrospectively in frozen specimens from 46 invasive breast tumors classified according to estrogen receptor (ER) and HER2 status. **Results:** All specimens were taken at diagnosis and validated for tumor content >50%. Twenty-nine were ER+, 17 were HER2+, 10 were triple negative (TN) tumors. Analyses were performed in triplicate from total protein extracts and were achievable in all specimens. P38MAPK was found to be expressed in all tumor specimen and significantly (P=0.002) overexpressed in ER+ tumors. P38MAPK was lower in TN tumors than in all others. The median expression of phosphorylated-P38MAPK was also higher in ER+ than in ER- tumors and lower in TN tumors than in all others. HER2 status had no influence on P38MAPK and p-P38MAPK expression. No variation in the phosphorylation rate of P38MAPK was observed in relation with ER, HER2 or TN status. Significantly higher (P=0.0048) expression of p-AKT tumors was observed in HER2+ tumors. No significant difference in p-MEK1, p-GSK3β and p-S6K expression was evidenced in any other comparisons based on ER and HER2 expression subtypes. **Conclusions:** Investigation of the expression of multiple phosphorylated signaling proteins can be used as molecular biomarkers for personalized targeted therapy. In ER+ invasive breast cancer, the overexpression of P38MAPK could serve as biomarker for evaluation of P38MAPK inhibitors.

PO10

DDB2 a new regulator of metabolism and cell death in human breast tumor cells

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Auteur présentant le résumé : Rémi KLOTZ

Résumé : Scientific background: The protein Damaged DNA Binding-2 (DDB2) is well known for its role in DNA repair by nucleotide excision repair. Interestingly, DDB2 is differentially expressed in breast cancer expressing the estrogen receptor alpha or not. Recent works performed in our laboratory showed a new role of DDB2 in the control of proliferation and invasive abilities in different breast tumor cells through its involvement in the transcriptional regulation of target genes. Two genes involved in tumorigenic processes, MnSOD (manganese superoxide dismutase) and IkBalpha (inhibitor alpha of Nuclear Factor-kappa B), have been found to be regulated by DDB2. In addition, transcriptomic analyses showed that several genes involved in cellular metabolism regulation seem to be modulated by DDB2. Our aim is now to focus on the effects of DDB2 expression on cellular metabolism and in the response of breast cancer cells to anticancer agents.
Material and Methods: Metastatic breast cancer cells, MDA-MB 231, expressing endogeneously low level of DDB2, have been used. In parallel, the gene encoding DDB2 was introduced experimentally in this model. To study the influence of DDB2 on the mitochondrial metabolism, we performed oxygraphic measurements. Moreover, the use of oligomycin, an ATPase inhibitor, was done to estimate the efficacy of the mitochondrial respiratory chain. In addition, we performed measurements of reactive oxygen species. Finally, cells were exposed to two anticancer drugs commonly used in the treatment of breast cancer: doxorubicin and 5-Fluorouracil and to one apoptotic agent, Tumor Necrosis factor alpha (TNFalpha). The sensitivity of cells to these agents was analysed by evaluating apoptosis in vitro and in vivo.
Results: Our results indicated that the overexpression of DDB2 leads to a respiratory chain dysfunction and an increase of the glycolytic pathway. Moreover, we observed an increased production of reactive oxygen species in these cells, compared to parental cells. As mitochondria are involved in cell death, we performed different experiments to evaluate the impact of DDB2 in the response to anticancer agents (Doxorubicin, and 5-fluorouracile) commonly used in the treatment of breast cancer. Interestingly, the cells exhibit a greater sensitivity to anticancer drugs when DDB2 is overexpressed. As these two agents are related to DNA damaged, we have also used other molecules, the apoptotic inducer, TNFalpha to precise the role of DDB2 on cell death. Similar results were obtained, thus demonstrating the influence of DDB2 overexpression in the response to cell death.
Conclusion: All together, our results indicate that DDB2 is a potential predictive marker of sensitivity to anticancer drugs and is of important clinical interest to breast cancer research. Finally, if the link between DDB2 and mitochondrial activity is confirmed, targeting mitochondria could be used as a therapeutic target in breast cancer

PO11

DIAGNOSIS OF HEPATOCELLULAR CARCINOMA IN CIRRHOTIC PATIENTS: A PROOF-OF-CONCEPT STUDY USING SERUM MICRO-RAMAN SPECTROSCOPY

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Auteur présentant le résumé : Imane TALEB

Résumé : Hepatocellular carcinoma (HCC) is the third most common cause of cancer death worldwide. The development of novel diagnostic methods is crucial to detect the tumor at an early stage when patients are eligible for curative treatments. The purpose of this proof-of-concept study was to determine whether micro-Raman spectroscopy applied to the serum of cirrhotic patients could be useful in distinguishing patients with and without HCC.
Patients and methods: Serum samples were collected from 2 groups of patients: cirrhotic patients with HCC (n=37) and without HCC (n=34). The micro-Raman spectra were acquired from dried serum drops and freeze dried serum in the point-mode with a LabRam microspectrometer (Horiba Scientific, France) equipped with 100x objective and a 785 nm laser. For each sample, 5 replicates were recorded at the centre and at the periphery, each during 40 s in the spectral range 600-1800 cm-1. Spectra were quality-tested and preprocessed (smoothing, baseline subtraction, vector normalization). Then, principal component analysis (PCA) was performed and the support vector machine (SVM) applied using the leave-one-out cross validation (LOOCV) procedure to classify the spectra into 2 classes of cirrhotic patients with and without HCC.
Results: Using PCA, the principal component score plots did not show any clustering tendency and did not allow separating spectra from cirrhotic patients with and without HCC. In contrast, SVM method using the LOOCV procedure was able to correctly classify the 2 groups of patients with a sensitivity between 81 and 92% and specificity between 82 and 92% for dried serum drop. Regarding freeze dried serum, this classification procedure gives a sensitivity between 86% and 95% and specificity between 82% and 89%. The use of the whole set of spectra rather than the mean or the median spectra did not improve the performance of the classifier.
Conclusion: The promising results obtained in this pilot study support the working hypothesis that serum micro-Raman spectroscopy may become a useful diagnostic tool to detect biomarkers in the field of cancer, as here for distinguishing between cirrhotic patients with and without HCC.
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PO14

IDENTIFICATION AND QUANTIFICATION OF TUMORAL CELLS (CSFTCs) IN CEREBROSPINAL FLUID OF PATIENTS WITH LEPTOMENINGEAL METASTASES.

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Résumé :
Background
The usual diagnostic methods of leptomeningeal metastases (LM) in CerebroSpinal fluid (CSF), i.e cytology and gadolinium enhanced MRI, lack both specificity and sensitivity. The Veridex CellSearch® technique which allows to quantify circulating tumour cells (CTCs) in peripheral blood is validated for follow-up and prognosis evaluation in various cancers. We adapted this technology to detect Tumour Cells (CSFTCs) and Melanoma Cells (CSFMCs) in CSF from cancer patients presenting with LM.
Methods
CSF samples from 80 patients with established or suspected breast cancer or lung cancer LM and/or melanoma LM were evaluated using usual criteria and an adapted CellSearch® Veridex technology. CSF was obtained at lumbar or ventricular sites. For CellSearch® analysis, 5 mL CSF samples were collected on CellSave® Preservative tubes at the time of sampling, shipped at room temperature and analyzed within 3 days after CSF sampling.
Results
Gold Standard cytological analysis on 1 to 10 mL CSF samples from patients with established LM sometimes allowed the detection but usually not quantification of TCs, even in optimal samples. CellSearch® analysis could not detect TCs or artefactual images in patients without established LM. In established LM, EpCAM+/cytokeratin+ or CD146+/HMW-MAA+ nucleated (DAPI+) cells were observed and enumerated with precision from one to up to 10 000 cells/mL. Their morphology could be readily appreciated on digital images galleries and was discriminant between breast and lung cancer samples. We coin the acronym CSFTCs for these cells. The detection of and follow-up CSFMCs (M for melanoma) is also possible. In CSF from lung cancer patients tumour cell aggregates previously described in blood as CTMs (Circulating Tumor Microemboli) could be seen.
Conclusions
This methodology, established on a limited volume of LCR compared to the Gold Standard and allowing delayed processing, could prove of great interest in the diagnosis and follow-up of various cancer patients with LM. A study is now ongoing as an ancillary study of the DEPOSEIN clinical trial (EudraCT 2010-023134-23) to confirm these data and to evaluate in sequential samples the effects of an intrathecal administration of the drug DepoCyte® for its clinical and biological efficacy. The reliability of the method also opens new fields of investigation for other biological fluids.

PO18

REVISITING TFF1 AND TFF3 AS BIOMARKERS IN BREAST CANCER

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Auteur présentant le résumé : Stéphanie DELPOUS

Résumé : Headlines scientific background. A better knowledge of mammary tumorigenesis allows the development of personalized patient care. For example, adjuvant chemotherapy is not justified for all patients. While it is beneficial for those at high risk of relapse, it might be detrimental for other patients. This therapeutic decision is based on several criteria and can be difficult in some cases. Therefore, it is necessary to develop new prognostic tools that predict recurrence in early stage breast cancer and chemotherapy benefit. TFF1 and TFF3 proteins represent potential prognostic markers. Indeed, TFF1 and TFF3 are two related proteins induced by estradiol, and present in some breast cancers. Studied in isolation, their value as prognostic markers remains controversial. Our objective is to evaluate the prognostic potential value of combined TFF1 and TFF3 dosages. Patients, materials and methods. The expression of TFF1 and TFF3 proteins was measured in a prospective series using a semi quantitative methodology. Western Blot (WB) analysis was performed in 200 primary breast tumors from patients who presented in the Hautepierre hospital of Strasbourg between October 14, 2011 and February 15, 2013. An immunoscore (WB) was established (0: negative, +: low, ++: medium, +++: high). The association between TFF1 and TFF3 expressions with various clinico-pathological features was determined. Probabilities of <0.05 were considered statistically significant. Results. Unexpectedly, there is not a complete association between TFF1 and TFF3 levels in breast cancers. Tumors TFF1 positives were strongly associated with the presence of estrogen receptor. Tumors double positives for TFF1 and TFF3 were associated with poor prognostic indicators (larger tumor size, higher grade and lymphovascular invasion). In contrast, tumors only positive for TFF1 were associated with good prognostic indicators. Conclusion and perspectives. This prospective study shows that in contradiction with some published reports, TFF1 and TFF3 are not systematically co-expressed in breast cancers and that they behave like independent markers. Of interest, this study suggests that TFF1-, TFF3- single and double positive tumor may distinguish several subtypes within ER-positive tumors. Indeed, TFF1 and TFF3 double positive tumors are associated with some indicators of poor prognosis. In addition, Smid et al (JCO 2006) found both genes in a gene signature for the prediction of subsequent metastasis to bone. To validate our results, we will extend our study by using a second methodology for the determination of TFF1 and TFF3 on breast cancers. Using the same series, TFF1 and TFF3 proteins will be measured by ImmunoHistoChimestry (IHC). The present study reinforces the view that TFF1 and TFF3 expression merit further evaluation as prognostic markers.

PO21

Vimentin expression predicts the occurrence of metastases in non small cell lung carcinomas

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Auteur présentant le résumé : Philippe BIREMBAUT

Résumé : Epithelial-to-mesenchymal transition (EMT) is believed to contribute to tumour invasion. Vimentin expression by carcinoma cells is a largely recognized marker of EMT. This study aimed at examining vimentin expression in non small cell lung carcinomas (NSCLC) by immunohistochemistry to evaluate potential correlations between vimentin expression and the differentiation status, the TNM stage and the outcome of the patients. 295 NSCLC including 164 squamous cell carcinomas (SCC), 108 adenocarcinomas (AC) and 23 other NSCLC carcinomas have been examined by immunohistochemistry. Vimentin was indeed detected in 145 cases (49.2%). It was principally present in isolated tumour cells and invasive clusters, particularly in cells at the tumour/stroma interface. Vimentin expression was significantly more expressed in large cell neuroendocrine, adeno-squamous and sarcomatoid carcinomas than in SCC and AC and was significantly associated with the differentiation status of carcinomas. The follow-up of 193 patients further demonstrated that an extensive expression of vimentin (>50% of tumour cells) was associated with the occurrence of metastases. In conclusion, our data demonstrate that vimentin expression is a frequent event in NSCLC and that its expression can be associated with a lack of differentiation and the occurrence of metastases.

PO33

BILE ANALYSIS USING FOURIER-TRANSFORM INFRARED SPECTROSCOPY COMBINED WITH CHEMOMETRICS FOR THE DIAGNOSIS OF MALIGNANT BILIARY STRICTURES: A PILOT STUDY IN 57 PATIENTS.

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Auteur présentant le résumé : Gérard THIEFIN

Résumé : The differential diagnosis between malignant and benign biliary strictures is a challenging task for clinicians. Clinical, biochemical and radiological characteristics are non-specific and tissue or cell diagnosis is difficult to obtain preoperatively. Given all these limitations, there is a need for the development of new diagnostic modalities. This study aimed at determining whether FTIR spectroscopy is potentially able to distinguish bile samples from patients with and without malignant biliary strictures.
Patients and methods: The study was performed in 19 patients with malignant biliary strictures and 38 with benign biliary diseases. Bile samples were collected during endoscopic retrograde cholangiopancreatography. After centrifugation, the supernatant was retrieved and subjected to lipid extraction. This allowed separating bile aqueous phase and bile organic phase. FTIR spectra (10 per sample) were acquired in the transmission mode on these fractions as well as the whole bile at a spectral resolution of 4 cm^{-1} using 32 scans, over the range 400-4000 cm^{-1}. Spectra were then quality-tested. After preprocessing by EMSC and principal component analysis, the support vector machine (SVM) classification was applied using the leave-one-out cross validation (LOOCV) procedure to classify the spectra into 2 groups corresponding to patients with and without malignant biliary strictures.
Results: 6.2% of raw spectra were discarded following quality tests. When applied to validated spectra from the whole bile, the aqueous and the organic phases, SVM method using LOOCV procedure correctly classified the two groups of patients with a sensitivity between 82% and 90% and a specificity between 85% and 100%. The overall accuracy rate was 84%-96%. Results obtained with spectra from the whole bile were not significantly different from those obtained with the spectra from the bile aqueous phase and the bile organic phase.
Conclusion: This pilot study suggests that FTIR spectroscopy combined with chemometrics can differentiate between bile samples from patients with malignant biliary strictures and those with benign biliary tract diseases. Separate analysis of bile aqueous and organic phases does not appear to significantly increase the diagnostic performance compared with analysis of the whole bile.
This study was funded by Reims University Hospital (AOL 2011). The IBiSA technological platform "Imagerie Cellulaire et Tissulaire » is gratefully acknowledged.

PO34

NANOS-3: A NEW BIOMARKER IN NON-SMALL CELL LUNG CANCER?

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Auteur présentant le résumé : Simon GRELET

Résumé : Lung cancer is the leading cause of death worldwide. The poor survival of lung cancer patients is mostly attributed to early metastasis and resistance to therapies. The metastatic progression of epithelial tumors is a complex process requiring tumor cell plasticity. In particular, the step of tumor cell invasion into surrounding stromal tissue is characterized by a dedifferentiation of tumor cells largely known to involve epithelial-mesenchymal transition (EMT). There is growing evidence that EMT increases resistance to conventional chemotherapy, radiotherapy as well as targeted therapy.
Nanos genes proteins comprise a small family of homologous genes coding for (CCHC)2 zinc finger proteins showing conserved evolutionary functions in germ cell development. We have previously reported that human Nanos-1 is repressed by E-cadherin and induces invasion of lung tumor cells by upregulating the matrix metalloprotease (MMP)-14. Our preliminary in vitro screening of NANOS-2 and -3 gene expression in various carcinoma cell lines revealed that Nanos-3 could play a role in the tumor invasion process during bronchopulmonary carcinoma progression.
Our study here showed that Nanos-3 cDNA overexpression in human lung carcinoma cell lines results in the acquisition of several features of EMT, including a morphological change from a cohesive epithelial shape to a more scattered fibroblast-like shape, downregulation of the epithelial markers E-cadherin and occludin, and overexpression of the mesenchymal markers vimentin and slug. These phenotypic changes are associated with increased invasive and migratory abilities correlated with an upregulation of MMP-14 and urokinase Plasminogen Activator (uPA) expression as demonstrating by western blot and zymography analyses. By using RT-PCR and RNA immunoprecipitation (RIP) analyses, we found that Nanos-3 regulates its targets through both transcriptional and post-transcriptional mechanisms and that mRNA of post-transcriptionally regulated targets are systematically co-immunoprecipitated with the protein Nanos-3. In silico and in vitro, by using bioinformatics predictions, subcellular fractionation and confocal laser scanning microscopy, we demonstrated that Nanos-3 is localized in both the cytoplasm and the nucleus with a high accumulation in the nucleolus. In vivo, western-blot analysis indicated that Nanos-3 is overexpressed in human non-small cell lung carcinomas (NSCLC) and correlates with the aggressiveness of cancer according to the TNM stage. By immunohistochemistry, Nanos-3 was detected in tumor cells, principally at the invasion front of tumor clusters. Moreover, overexpression of Nanos-3 in NSCLC is correlated with a decrease of the overall survival of the patients.
These results demonstrating a new role for Nanos-3 in the acquisition of an invasive phenotype by lung tumor cells suggest that Nanos-3 could be a biomarker in NSCLC.

PO38

INTERMEDIATE-CONDUCTANCE CA2+-ACTIVATED KCa3.1 CHANNEL REGULATES BREAST CANCER MIGRATION AND INVASION, IN VITRO AND IN VIVO, THROUGH A CLOSE CORRELATION WITH TRPC1

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Auteur présentant le résumé : Anne-Sophie AY

Résumé :
Scientific background: Breast cancer (BC) is the most frequently occurring cancer in women and has the highest rate of mortality. Both TRPC1 and the intermediate-conductance Ca2+-activated K+ channel (KCa3.1) have already reported to control BC cell proliferation by regulating cell cycle progression. However, the mechanism(s) by which these channels control migration remains unknown. Both channels regulate the entry of Calcium (Ca2+). Indeed, TRPC1 is a component of Store Operated Channels (SOCs) that permit Ca2+ entry and KCa3.1, by inducing hyperpolarization of the membrane potential, increases the Ca2+ entry.
Material and methods: All experiments were performed in the highly invasive MDA-MB-231 cell line. TRPC1 and KCa3.1 expression was silenced by using specific siRNA and siRNA efficiency was verified using both Q-PCR and western blot. Migration was studied using Boyden's chamber and videomicroscopy. Physical interaction between TRPC1 and KCa3.1 was analyzed using protein immunoprecipitation (PI) and confocal microscopy. Calcium imaging was performed to determine Ca2+ entry. Expression of both channels in BC human tissue was performed by Immuno-histochemistry and mouse experiments were performed on nude mice by xenograft. MDA-MB-231 cells expressing luciferase and transfected with siTRPC1, siKCa3.1 or siControl (si-CTL), were injected in tail vein and TRPC1 and KCa3.1 siRNA were injected twice a week. Metastases formation and size were monitored twice a week by bioluminescence.
Results: Both TRPC1 and KCa3.1 are localized in the front area of migrating cells with some co-localisation (about 25 %). Using PI analysis, we show that TRPC1 and KCa3.1 proteins interact likely in lipid raft. Down-regulation of TRPC1 or KCa3.1 induced a strong decrease of migration, velocity and translocation with a slight increase in viability in TRPC1 silenced cells. Interestingly, silencing both channels showed non additive effect suggesting employment of the same pathway. Concomitantly, knockdown of KCa3.1 or TRPC1 reduced Ca2+ entry. Both TRPC1 and KCa3.1 proteins were preferentially expressed in human BC tissues (n=60). Moreover, we found a higher TRPC1 and KCa3.1 expression in ER- Invasive Ductal Adenocarcinoma tissues and lymph nodes than in the non-invasive tumoral samples. Finally, metastases appear at week 5 in si-CTL mouse. At 8 weeks, we showed the development of 5 to 10 metastasis in mouse injected with si-CTL while, si-TRPC1 and si-KCa3.1 mouse present between 0 and 6 and 0 and 2 metastasis respectively. Moreover, metastasis size is smaller in si-KCa3.1 mouse but bigger in si-TRPC1 mouse corresponding with viability results in vitro.
Conclusions: Our results highlight TRPC1 and KCa3.1 channels in breast cancer metastasis and we propose these two proteins as prognosis markers and potential therapeutic targets in treatment of human invasive BC.

PO45

PRÉVALENCE OF BRAF AND CKIT MUTATIONS IN TUMOR SPECIMENS FROM PATIENTS WITH MELANOMA IN CHAMPAGNE-ARDENNE

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Auteur présentant le résumé : Olivia BEAUDOUX

Résumé : Background : Recently, oncogenic mutations involving BRAF and cKIT genes have been identified in melanoma. These mutations are already being successfully exploited as therapeutic targets for treatment or inclusion in clinical trials of metastatic melanoma patients. The aim of this study was to determine the incidence of BRAF and cKIT mutations in tumor samples addressed to the PGMCCA platform.
Patients and methods : From January 2011 to December 2012, tumors of 176 patients (91 women and 85 men) were received. 87 specimens were collected from cutaneous tissues and 89 from other sites (among 39 lymph nodes, 12 mucosal tissues, 7 stomach, 6 liver, and 5 lung). 55 samples were obtained by biopsy, 120 by surgery and one by cerebrospinal ponction. 103 were collected from metastatic sites and 73 from primitive tumors. Median cell tumor percentage was 70% +/-25%.
Genomic DNA was prepared from formalin-fixed paraffin-embedded (FFPE) sections of tumor specimens and extracted by Qiagen® manual extraction or Maxwell16™ system. BRAF V600E was screened with a TaqMan PCR assay, combined with direct Sanger sequencing in exon 15. cKIT gene was analysed by direct sequencing in exons 11, 13, 17 and 18. As BRAF and cKIT are mutually exclusive oncogenic mutations, only BRAF wild-type tumors were examined for cKIT mutations.
Results : 61 mutations in BRAF gene were detected in 170 patients (35.8%) : 22 women (22/91= 24.1%) and 39 men (39/85= 45%). Of the 61 mutations, 52 (85.2%) were V600E mutations, 6 (9.9 %) V600K and 3 (4.9%) uncommon mutations (V600R, V600E2 and K601E). Direct sequencing and TaqMan PCR were concordant in all V600E positive samples. Uncommon mutations were identified by direct sequencing only. Six samples were invalid because of DNA amplification failure (6/176=3.4%).
Six mutations in cKIT gene were detected in 82 patients (7.3%) : 3 in exon 11, 1 in exon 13, 1 in exon 17 and 1 in exon 18. Twenty seven samples were invalid (27/109=24.8%). Invalid results were mainly explained by poor DNA quality or quantity (15 samples presented tumor cell percentage < 15%), fixation (2 samples fixed with Bouin liquid), or pigmented samples (n=10).
Conclusion : the incidence of BRAF and cKIT mutations observed in this cohort is comparable to national data. In 2011, the National Cancer Institute (INCa) reported mutation rates at 37.6% for BRAF and 3.6% for cKIT.

PO46

EVALUATION OF COMPETITIVE ALLELE SPECIFIC TAQMAN REAL-TIME PCR FOR BRAF V600E MUTATION DETECTION IN CLINICAL FORMALIN FIXED PARAFFIN EMBEDDED SAMPLES OF MALIGNANT MELANOMA

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Auteur présentant le résumé : Olivia BEAUDOUX

Résumé : Background : BRAF is the most frequently mutated oncogene in melanoma with BRAF V600E mutation accounting for 92% of all BRAF variants. The availability of BRAF V600 inhibitors for the treatment of melanoma has created the need for validated mutation detection assays to select patients. The aim of this study was to evaluate BRAF genotyping by a high sensitive method, CAST-PCR™ (Competitive Allele Specific TaqMan® PCR), focusing on BRAF V600E (c.1799T>A, p.V600E).
Method : CAST-PCR™ (Life Technologies) is a real time quantitative Clamp-based PCR, based on Competitive Allele Specific hydrolysis probes TaqMan® with the use of a locked nucleic acid probe and an allele specific primer.
Material / Study design : Analytical sensitivity was examined by dilutions of formalin fixed paraffin embedded (FFPE) titrated standard of mutant V600E DNA (Horizon Diagnostics®). Diagnostic sensitivity and specificity were analysed using 28 clinical anonymous FFPE melanoma tissues. Results were compared to those obtained by two other methods (Sanger sequencing and hydrolysis (TaqMan®) PCR). DNAs were extracted by QIAamp® DNA Mini Kit (Qiagen). Robustness was tested using quantity variations (20 to 1 ng) of BRAF homozygous V600E human cell line DNA (SK-MEL-28 (ATCC® HTB-72™)) and high pigmented clinical samples (n=2).
Results : Analytical sensitivity was 0.1% in FFPE matrix.
Diagnostic sensitivity and specificity : of the 29 cases included, 13 were wild-type (WT) for BRAF, 13 had V600E mutations, and 3 had others V600 mutations. Complete concordance was reported in 28/29 cases. One case showed different results between the three assay types (no amplification by CAST-PCR™, V600E by Sanger sequencing and TaqMan® PCR), explained by poor quality DNA.
Robustness : DNA quantity was reduced at 1 ng with similar amplification and results than with 20 ng (recommanded quantity). Pigmented samples (n=2) were amplified without trouble.
Conclusion : CAST-PCR™ assay shows an analytical sensitivity <1% which is higher than TaqMan® (10%) and Sanger sequencing (15 at 20%) sensitivity. It assay allows the sensitive, specific and robust measurement of BRAF V600E in FFPE melanoma samples. Some authors reported that CAST-PCR™ with very high sensitivity has potential applications in plasma mutated DNA detection. As this event occurs early in melanoma progression, the quantification of BRAF-mutated alleles in plasma may represent a useful biomarker for non-invasive prediction and evaluation of response to BRAF inhibitors.

PO2

INTRATUMORAL DISTRIBUTION OF THE BIOMARKERS HER2, PS2, MIB1 AND HORMONE RECEPTORS IN BREAST CANCER ACCORDING TO AGE AND SBR

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Auteur présentant le résumé : Hayette Sénia BENSABER

Résumé : The objective of our work is to study the impact of aging and the grading on the intratumoral heterogeneity within a sample of mammary infiltrating ductal carcinomas with the aim of bringing participation to the improvement of the diagnosis and the evaluation of the individual prognostic of patients. It keeps pace with a better comprehension of cellular dynamic mechanisms in human oncogenesis.
Its age-specific incidence profile rises until menopause and increases more slowly thereafter, reflecting the superimposition of early-onset and late-onset breast cancer rates. (THUN and JEMAL, 2006). The heterogeneity is expected by the expression variability of C-erbB-2 proto-oncogene, PS2 estrogen-regulated protein, Ki67 cellular proliferation marker and estrogen and progesterone receptors. Image acquisition and simultaneous visualization of labels were assessed by microscopy. Images were treated by MATLAB 7.6 software. A systematic sampling of the histological cuttings was realized for intratumorale heterogeneity estimation. According to 'age' criteria, the majority of assessed patients belonged to the age group 40 years and older (87.7%). Among the clinicopathological parameters, the right breast is more affected in young women (66.7%) with more frequent tumor size >4cm (66.7%). The score '0' is mainly present in the two groups; unlike C-erbB-2 label where the trend is more towards the score '>0' in young women (55.6%). The labeling index (LI) was used as a parameter to evaluate spatial distribution of surexpression markers. For every label, the LI is found at variable rates in various microscopic fields of the same tumor belonging to the same age group of the patients. These results confirm the intratumor heterogeneity profile and show that the labeled cells do not follow the same distribution in both groups. The analysis of labels expression variability estimated by the coefficient of variation (CV) revealed very scattered values demonstrating an intratumor heterogeneity but without discerning significant difference between both groups; the minimal value is found in the case of the expression of progesterone receptors ($p=0.221$). The Pearson test of correlation revealed no relation between the CV and the age also ($0.39>r>-0.06$). By considering the SBR grade, it seems that heterogeneity does not seem influenced by the grading. The analysis of variance reveals no significant difference at 5% level between SBR 2 and SBR 3 grades for all the markers ($0.84>p>0.12$). In conclusion and according to the methods used in this study, it seems that no marker managed to demonstrate a relation between intratumor heterogeneity and tumor progression. However, the conflict in the markers expression found in patients can explain known therapeutic treatments failure.
Keywords : Breast cancer, heterogeneity, age, SBR, Biomarkers HER2, PS2, MIB1 and Hormonal receptors.

PO8

ELASTIN PEPTIDES: AN INTERFACE BETWEEN CANCER AND CONTROL OF GLUCOSE HOMEOSTASIS

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Auteur présentant le résumé : Beatrice ROMIER-CROUZET

Résumé : Introduction: In Europe, more than 55 million patients suffer from type 2 diabetes (T2D). The WHO estimates that in 30 years, more than 66 million people will be affected by this disease. Real public health problem, T2D is a major risk factor for cardiovascular diseases such as atherosclerosis characterized by lipid accumulations and elastin degradation into elastin peptides (EP) and favored by the ageing process. The EP that have been linked to cancer progression especially melanoma growth, influence the cell physiology by activation of the elastin receptor complex, composed of elastin binding protein (EBP), cathepsin A (PPCA) and neuraminidase-1 (Neu-1). Moreover, in vitro, Neu-1 modulates the insulin receptor (IR) activation, regulator of the glucose homeostasis.
The objective of this study was to establish in mice, the functional link between EP and insulin sensitivity and the consequences on glucose and lipid utilizations by tissues.
Methods: To mimic the effects of EP accumulation during ageing, we performed in normal mice, chronic i.v. injections of either a mixture of active EP (produced by hydrolyzis of elastin) or a unique and specific sequence of active EP (VGVAPG). Glucose tolerance tests and insulin sensitivity tests were performed.
Results: With these models, we showed that EP injections induced a glucose intolerance associated with insulin resistance and lipid storages in tissues (liver, muscle and adipose tissue) . At cellular level, we observed a diminution of IR activity which, could be explained, at molecular level, by the interaction of Neu-1 with IR, inducing a sialic acid reduction on IR.
Conclusion: The IR signaling alteration by EP suggested that the elastin degradation may be one of the key factor implicated in the appearance of T2D which is more prevalent in ageing subjects.

PO27

IMPACT OF ANTICANCER TREATMENTS ON TUMOR MICROVESICLES SHEDDING BY MALIGNANT GLIOMAS CELLS

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Auteur présentant le résumé : Haixia DING

Résumé : Aims: Glioblastoma (GBM) is the most lethal of all human tumors. It has been proved that GBM cells release tumor microvesicles (TMVs) to disseminate information towards many different cells present in their microenvironment. These TMVs were able to interact with these neighboring cells and modify their phenotypes, thus participating in the tumor progression. After surgical resection, the concomitant administration of RT and temozolomide (TMZ), followed by adjuvant TMZ is the first-line therapeutic standard for GBM. The present study was designed to investigate in vitro the impact of anticancer treatments, especially RT and TMZ on the release of TMVs.
Materials and methods: Three GBM cell lines (U87, U373 and T98G) were seeded at 10 000 cells/cm2 and were grown for 48h. For RT study, cells were X-irradiated at 2 and 10 Gy with a Pantak Therapax SXT 150 apparatus. For TMZ study, cells were exposed to 100µM of TMZ. Cell culture media were collected at different time after treatment (2h, 24h, 48h post-RT and 4h, 24h for TMZ) and centrifuged twice at 2600g during 15 min. TMVs quantifications were performed by flow cytometry analysis using calibrated beads (Megamix®, Stago): events were counted during 300 sec with Particle Count Standard (Sure Count, Polysciences). Cell viability was assessed by cell counting. The effect of released TMVs on the global behavior (proliferation, adhesion, cell death) of HUVEC is investigated using the xCELLigence system.
Results: Forty-eight hours after 10Gy-irradiation, whatever the cell line studied the TMVs/cell release was increased, while treatment led to a cell number decrease of about 60%. In this case, 2-fold more TMVs/cell was found in culture media as compared to non-irradiated cells. In U87 cells, a peak of TMVs/cell release was noticed 2h after RT.
In T98G and U87, TMZ exposure induced a 1.5-fold increase in TMVs release 24h post-treatment while no effect on cell viability was observed. For U373 cell line, a similar effect was observed 4h post-treatment. The evaluation of TMVs effect on HUVEC by xCELLigence is ongoing.
Conclusion: Irradiation and TMZ increased the release of TMVs into culture media by glioblastoma cells. The appearance time of peak of release depends on the treatment type. The effect of TMVs on HUVEC is under investigation.

PO40

OVEREXPRESSION OF THE THROMBOSPONDIN-1 RECEPTOR CD47 CONTRIBUTES TO RESISTANCE OF HUMAN THYROID CARCINOMA FTC-133 CELLS TO DOXORUBICIN-INDUCED APOPTOSIS

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Auteur présentant le résumé : Hamid MORJANI

Résumé : Several studies have shown recently that the extracellular matrix (ECM) contributes to a new form of de novo resistance to chemotherapy, called environment-mediated drug resistance (EMDR). Two forms of EMDR have been described: soluble factor-mediated drug resistance (SFM-DR) and cell adhesion-mediated drug resistance (CAM-DR).
Thrombospondin-1 (TSP-1) which is a well known antiangiogenic factor via induction of apoptosis in endothelial cells via its N-terminal receptor CD36, has been described recently by our group and other authors as a SFM-DR able to modulate tumour cell response to chemotherapy via its interaction with its C-terminal receptor CD47. Our group has reported recently that doxorubicin is able to induce apoptosis in thyroid carcinoma FTC-133 cells via ceramide de novo synthesis (1) which triggers JNK pathway and caspase-3 activation (2). Doxorubicin-induced apoptosis was accompanied by a downregulation of TSP-1 expression. The addition of exogenous TSP-1 protected FTC-133 cells against apoptosis and involves its interaction with its receptor CD47 (3).
In order to study the involvement of TSP-1/CD47 interaction in resistance to chemotherapy, we have established a resistant FTC-133 cell line in the presence of 400 nM doxorubicin (FTC-133R). FTC-133R cells were 15-fold resistant to doxorubicin and showed a significant decrease in nuclear uptake of the drug, when compared to FTC-133 cells. Caspase-3 activation following doxorubicin treatment was significantly reduced in FTC-133R cells. These cells overexpressed MDR1 gene and its ABC product the P-glycoprotein. Verapamil was able to restore nuclear uptake of doxorubicin and sensitivity of FTC-133R cells to the drug-induced apoptosis. Analysis of TSP-1 expression showed similar levels of TSP-1 mRNA in FTC-133 and FTC-133R cells. However, mRNA expression of its C-terminal receptor CD47 was significantly increased in FTC-133R cells. Co-treatment of FTC-133R cells with verapamil and doxorubicin induced a decrease in CD47 mRNA expression. Moreover, transient knockdown of CD47 in FTC-133R cells using siRNA strategy positively regulates doxorubicin-induced JNK activation and sensitized these cells to drug-induced apoptosis. These data show that in addition to P-glycoprotein overexpression, CD47 is able to contribute to protection of FTC-133R cells against doxorubicin-induced apoptosis.
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PO41

EXTRACELLULAR MATRIX AGING ACCELERATES THE PROLIFERATION OF HT-1080 FIBROSARCOMA CELLS VIA ERK/MAPK PATHWAY AND P21CIP1 DOWNREGULATION

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Auteur présentant le résumé : Charles SABY

Résumé : Several studies have recently shown the role of extracellular matrix (ECM) in the modulation of the cell response to chemotherapy (1). However, the effects of ECM aging on proliferation and/or sensitivity of tumor cells to chemotherapy are not well established.
In the present study, we propose to investigate the effects of type I collagen aging, one of the ECM components, on the proliferation of HT-1080 fibrosarcoma cells in two- and three-dimensional culture models. To this end, rat tail type I collagens were prepared from new born (10 days), adult (2 months) and old (2 years) animals, as already described (2). First, we characterized the advanced glycation end products (AGE) levels of different collagens using SDS-PAGE electrophoresis and fluorescence spectroscopy. A significant increase in AGE level has been observed in collagen extracted from adult and old rats, with significantly higher levels for the former compared to the latter. In 3D matrix culture models, collagen from old rats was able to induce 2 to 3-fold increase in proliferation rate of HT-1080 cells. However, this effect was not observed in 2D coating culture models. Western-blot analysis showed MAPK/ERK pathway activation but neither PI3K/AKT nor JNK pathways. Collagen from old animals decreased expression of the CDK kinase inhibitor p21CIP1. The ERK/MAPK inhibitor U0126 decreased the cell proliferation rate in the presence of collagen from old animals. However, antibodies directed against alpha2/beta1 integrin subunits and knockdown of their mRNA using the siRNA strategy did not affect the cell proliferation rate in the presence of collagen from old animals.
Taken together, our data suggest that ECM aging regulate positively tumor cell growth. However, this effect is not alpha2/beta1 integrin-dependent. For future studies, it will be interesting to investigate whether the receptor for AGE (RAGE) is implicated in the increase in cell proliferation in the presence of old collagen.
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PO42

ELASTIN PEPTIDES SENSITIZE HUMAN BREAST CARCINOMA MCF7 CELLS TO DOXORUBICIN-INDUCED APOPTOSIS

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Auteur présentant le résumé : Hassan EL BTAOURI

Résumé : Several studies have shown recently that the extracellular matrix (ECM) contributes to a new form of de novo resistance to chemotherapy, called environment-mediated drug resistance (EMDR). However, some of the ECM components are able to sensitize tumor cells to the cytotoxic effect of anticancer drugs. TGF‑1 is able to sensitize ovarian carcinoma cells to paclitaxel (1). Thrombospondin-1 (TSP-1), which has been shown to protect thyroid carcinoma cells against doxorubicin-induced apoptosis (2), is able to sensitize prostate carcinoma cells to taxol (3).
A recent work from our laboratory has shown that elastin peptides are able to protect human fibroblasts against C2-ceramide-induced apoptosis (4). Elastin peptides treatment leads in this case to activation of the anti-apoptotic pathway Pi3K/Akt. Here, we demonstrate that elastin peptides are able to sensitize the human breast carcinoma MCF7 cells to doxorubicin-induced apoptosis. Indeed, doxorubicin induced apoptosis in MCF7 by triggering reactive oxygen species (ROS) accumulation, JNK pathway activation and caspase activation. Co-treatment with elastin peptides positively regulates doxorubicin-induced JNK activation and induced an increase in doxorubicin-induced apoptosis. This effect implicated the interaction of elastin peptides with elastin-binding protein (EBP) receptor which activates neuraminidase and induced lactosylceramide synthesis. Neuraminidase inhibitor DANA and the antioxidant N-acetyl-cysteine were able to abrogate these effects.
These data show, in addition to TGF‑1 and TSP-1, that elastin peptides can mediate chemotherapy sensitivity. As elastin peptides are ECM components, activating peptides or antibodies that mimic their action may be a strategy for modulation of response of breast carcinoma to chemotherapy.
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PO43

LUMICAN ANTI-MIGRATORY EFFECT ON MELANOMA CELL MIGRATION : NEW MEDIATORS IDENTIFIED

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Auteur présentant le résumé : Stéphane BREZILLON

Résumé : Lumican is a member of a small leucine-rich proteoglycan family and is secreted to extracellular matrix. In skin, lumican is a glycoprotein of 57 kDa. Apart from its structural function in the control of collagen fibril assembly, lumican presents potent anti-tumour properties. Previous works from our group showed that lumican core protein was able to inhibit melanoma cell migration *in vitro*. Moreover, lumican was shown to inhibit primary tumour progression and lung metastasis *in vivo*. Integrin α2β1 was characterized as a direct lumican receptor on melanoma cells. In parallel, an inhibition of the phosphorylation of focal adhesion kinase (FAK) was observed. In this study, we examined the effect of recombinant glycosylated 57 kDa lumican on melanoma growth and migration *in vitro* and partially characterized its mechanism of action.
The presence of glycosylated lumican in culture media significantly decreased growth of the different melanoma cell lines tested (A375, B16F1, HT144 and SK-MEL28). The time necessary for melanoma cell spreading was significantly shorter in presence of lumican in comparison to control. Moreover, addition of recombinant lumican inhibited the migration of these cells. Among the four melanoma cell lines tested, only one (A375 cells) expressed the α2β1 integrin, the earlier characterized lumican receptor. This result suggests that there are other receptors involved in lumican effect on melanoma cells. Results from phospho- receptor tyrosine kinase array showed that lumican decreases the phosphorylation of several receptors, mainly EGFR, MER, EphB2, EphB6, ROR and Tie in B16F1 cells. To characterize the mechanisms involved in the inhibition of cell migration by glycosylated lumican, the status of the phosphorylation/dephosphorylation of proteins in B16F1 cells was examined. An inhibition of the phosphorylation of AKT, β-catenin, p130CAS, all proteins involved in signal transduction, were found. Altogether, our results put a new light on mediators and mechanisms involved in anti-tumour effect of lumican.

PO47

A LEUCINE-RICH REPEAT 9 – DERIVED PEPTIDE FROM HUMAN LUMICAN, LUMCORIN, PREVENTS MELANOMA CELL GROWTH AND MIGRATION

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Auteur présentant le résumé : Katarzina PIETRASZEK

Résumé : Lumican, a small leucine-rich proteoglycan of the extracellular matrix, presents potent anti-tumour properties. Previous works from our group showed that lumican was able to inhibit melanoma cell migration and tumor growth *in vitro* and *in vivo.* Melanoma cells are capable to adhere to lumican, resulting in a remodeling of their actin cytoskeleton and preventing their migration. In parallel, an inhibition of the phosphorylation of focal adhesion kinase (FAK) was observed. In addition, we identified a sequence of 17 amino acids (aa) within the lumican core protein, named lumcorin, which was able to inhibit cell chemotaxis and reproduce anti-migratory effect of lumican *in vitro* (Zeltz et al., 2009). The aim of the present study was to characterize the antitumor mechanism of action of lumcorin.
Lumcorin significantly decreased the growth in soft agar of colonies of two melanoma cell lines - B16F1 cells (mice melanoma cell line) and SkMel-28 (human melanoma cell line) in comparison to control. Addition of 100µM lumcorin to serum free medium significantly inhibited B16F1 and SK-MEL28 cell migration. To characterize the mechanisms involved in the inhibition of cell migration by lumcorin, the status of the phosphorylation/dephosphorylation of proteins was examined. Lumcorin inhibited FAK phosphorylation in B16F1 cells. Since cancer cells have been shown to migrate and to invade by mechanisms that involve matrix metalloproteinases (MMPs), the expression and activity of MMPs in B16F1 cells were analyzed. The presence of lumcorin induced an accumulation of an intermediate form of MMP-14 (~59kDa), and inhibited MMP-14 activity. Altogether, these results suggest that lumcorin inhibits melanoma cell migration by involvement of two simultaneous mechanisms: inhibition of phosphorylation of specific proteins and decrease of MMP14 activity.

PO49

TYPE I COLLAGEN PROMOTES RESISTANCE AGAINST DOXORUBICIN-INDUCED APOPTOSIS IN HUMAN FIBROSARCOMA HT-1080 CELLS

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Auteur présentant le résumé : Nicolas ARNAUD

Résumé : *De novo* or acquired anticancer drug resistance is one of the major problems in chemotherapy. Several studies have recently shown that the extracellular matrix (ECM) contributes to a new form of *de novo* resistance to chemotherapy, called environment-mediated drug resistance (EMDR). Two forms of EMDR have been described: soluble factor-mediated drug resistance (SFM-DR) and cell adhesion-mediated drug resistance (CAM-DR). In this study, we investigated the mechanism by which type I collagen promotes a CAM-DR doxorubicin resistance.
We show that the 2-dimensional type I collagen coating (2D) microenvironment protects fibrosarcoma HT-1080 cells against the cytotoxic and apoptotic effects of the topoisomerase II inhibitor doxorubicin. Collagen at 5µg/cm^2 density inhibited significantly doxorubicin-induced cytotoxicity as evaluated by cell counting. Moreover, collagen coating protected HT-1080 cells against the cytotoxic effect of doxorubicin in a density-dependent manner (optimal coating, 80 µg/cm^2). Microspectrofluorometric analysis of doxorubicin-treated HT-1080 nuclei showed clearly that collagen coating did not affect nuclear accumulation of the drug. In the same conditions, collagen coating protected HT-1080 cells against the cytotoxic effect of the topoisomerase I inhibitor camptothecin, whereas no protection was observed in the presence of the nucleoside analogue gemcitabine and the mitotic inhibitor taxol.
Morphologic analysis of HT-1080 nuclei following Hoechst 33342 staining and fluorescence microscopy analysis revealed that collagen coating decreased the number of apoptotic cells after doxorubicin treatment. Flow cytometry detection of phosphatidylserine exposure analyzed with annexin V confirmed Hoechst 33342 staining data and showed that collagen coating decreased doxorubicin-induced phosphatidylserine exposure. Cell cycle analysis showed that in the absence of collagen, doxorubicin induced an irreversible arrest in the G_2/M phase. However, collagen coating did not alter the cell-cycle distribution in G2/M fraction after doxorubicin treatment. Finally, doxorubicin-induced apoptosis was also monitored by analysis of caspase-3/7 activation. Doxorubicin was able to induce a high level of caspase-3/7 activity in the absence of collagen and this activity was significantly reduced in collagen coating conditions.
Our study identifies type I collagen as an important survival factor in topoisomerases inhibitors-induced apoptosis and suggests it as a factor contributing to the generation of drug resistance.

PO1

GENERATION OF BI-SPECIFIC CYTOTOXIC T LYMPHOCYTES (CTL) AGAINST EPSTEIN-BARR VIRUS AND ADENOVIRUS

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Auteur présentant le résumé : Yingying WANG

Résumé : Objectives: Human Adenovirus (ADV) and Epstein Barr Virus (EBV) infections or associated diseases are serious complications following allogeneic hematopoietic stem cell transplantations which are responsible for significant morbidity and mortality. Although available anti-viral drugs as cidofovir (ADV) and Rituximab (EBV), their efficiency is impaired in absence of specific immune reconstitution and toxicity remains a concern. Furthermore, adoptive cellular immunotherapy with ADV or EBV cytotoxic T cells (CTL) has proven safety of use and efficiency as it restored specific immunity. The concern is now to develop such an immunotherapy against the 2 viruses simultaneously. We focused on the generation of anti-ADV/EBV bi-specific T cells and the comparison with the mono-specific T cells in respect of enrichment, efficiency and toxicity. Such a comparative study was only reported once (Khanna et al, 2011).
Methods: Peripheral blood mononuclear cells (PBMC) from 3 donors presenting a cellular immune response against ADV and EBV were stimulated with ADV or EBV antigens or both. Immunomagnetic selection based on IFN-gamma secretion was performed and functional assays were carried out with the expanded cells.
Results : After immunoselection, a mean of 62.3+-18.08%, 39.9+-25.54% and 60.6+-20.25% CD4+ secreting IFN-gamma and 33.1+-20.46%; 26.3+-32.29% and 60.7+-20.55% of CD8+ secreting IFN-gamma were recovered for ADV-CTL, EBV-CTL and ADV/EBV-CTL, respectively. Virus-specific T cells were expanded in vitro and their ability to secrete IFN-gamma and to proliferate after restimulation with virus antigens was confirmed. A specific lysis for EBV-CTL and ADV/EBV-CTL was observed against autologous target cells pulsed with EBV peptide pools (59.3+-22.5% and 43.7+-32.6, respectively) and a lower lysis for ADV-CTL and ADV/EBV-CTL against autologous target cells loaded with PepT-ADV (31.0+-4.0% and 14.0+-6.1, respectively). A reduced alloreactivity of bispecific and monospecific T cells against third-party donor mononuclear cells, compared to the PBMC before selection was observed.
Conclusion: Despite a good enrichment in CD4 IFN-gamma and CD8 IFN-gamma T cells, bi-specific CTL showed a loss of cytotoxicity against ADV presenting targets compared with ADV mono-specific CTL. Although we cannot exclude antigenic competition during multi-specific CTL stimulation, our data seem to incriminate the amplification step required to perform functional assays.

PO13

PHASE I STUDY OF OM-174, A LIPID A ANALOGUE, WITH ASSESSMENT OF IMMUNOLOGICAL RESPONSE, IN PATIENTS WITH REFRACTORY SOLID TUMORS.

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Auteur présentant le résumé : Catherine PAUL

Résumé : Background: Lipids A, the lipophilic partial structure of lipopolysaccharides, induce regression of several tumor types in animal models. Rather than exerting direct cytotoxic effect, these compounds trigger the immune system which in turn stimulates secretion of cytokines, and activates the inducible nitric oxide synthase, as well as immune cell infiltration of tumors. OM-174 is an analogue of lipid A with dual action on toll-like receptors 2 and 4. In an experimental model of peritoneal carcinomatosis induced in BDIX rats by intraperitoneal injection of syngeneic PROb colon cancer cells, it induced a complete regression of tumors. The present phase I trial was conducted to determine the maximum tolerated dose, the recommended phase II dose and biological response associated with OM-174 administered as intravenous infusion.
Methods: Patients received OM-174 twice weekly for a total of 5, 10 or 15 injections of either 600, 800 or 1000 µg/m². Blood samples for pharmacokinetic analysis and cytokine dosages were collected. NK cells activity and toll-like receptors 4 polymorphism analysis were also performed.
Results
Seventeen patients were included. The highest dose administered was 1000 µg/m² repeated in 10 injections. The most common toxicities were a chills, fever, nausea/vomiting, diarrhea, fatigue and headache. No patient experienced haematological side effects. As no dose limiting toxicity was observed, despite a grade 3 respiratory complication, the maximal tolerated dose and recommended dose were not established. Three patients exhibited disease stabilization with a mean duration of 4 months. Pharmacokinetic profile of OM-174 was characterized by a low distribution volume and clearance. Analysis of TLR 4 polymorphysm showed that most (16/17) patients carried the wild type alleles. A progressive increase in NK cell number and activity was observed only in patients receiving 1000 µg/m2 of OM-174. A peak of IL 8 and IL-10 concentrations were observed after each OM-174 injection. Peaks of TNF-alpha and IL-6 concentrations were detected after the first infusion and decreased progressively suggesting tolerance.
Conclusion: OM-174 therapy was well tolerated at biologically active concentrations. Whereas the recommended dose was not determined, further studies are planned in combination with chemotherapy as animal models suggest a strong synergistic antitumor effect.

PO15

INFRARED SPECTRAL IMAGING; A NEW AUTOMATED DIAGNOSTIC TOOL FOR COLON CANCER

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Auteur présentant le résumé : Jayakrupakar NALLALA

Résumé :
Scientific Background: Novel methods are the need of the hour that could complement the 'gold standard' histopathology for cancer diagnosis. In this perspective, biophotonic approach of infrared (IR) spectral micro-imaging is one of the candidates, as it provides spectral fingerprint of cell and tissue biochemistry in a non-destructive and label-free manner. This ability has been exploited: 1) to develop a new concept of spectral bar-coding for rapid characterization of biochemical alterations between normal and tumoral epithelial components of colonic tissues, and 2) to identify spectral signatures for colon histology in order to develop a prediction model comprising potential diagnostic markers for rapid and automated colorectal cancer diagnosis.
Materials and Methods: Ten frozen colon tissue samples (five tumoral and non-tumoral pairs from five patients), and sixty-eight colonic samples (39 tumoral and 29 non-tumoral) from 32 patients in the form of paraffinized tissue arrays were imaged using IR spectral micro-imaging in a non-destructive manner. In case of paraffinized tissues, in order to avoid chemical deparaffinization, a mathematical deparaffinization based on extended multiplicative signal correction (EMSC) was implemented to neutralize the spectral interferences from paraffin. The spectral images were processed by a multivariate clustering method to identify the histological organization in a label-free manner.
Results: In the first part, the spectral information from the epithelial components of the frozen tissues was automatically recovered on the basis of the intrinsic biochemical composition, and compared using a statistical method (Mann-Whitney U test) to construct spectral barcodes specific to each patient. In the case of paraffinized tissue arrays, an LDA based robust prediction model (comprising 86802 spectra, constructed from 9 samples, and tested on 59 unknown samples involving a huge bank of 3620287 spectra) showed 100 % sensitivity for malignancy, while 10 out of 29 non-tumoral samples were identified as having tumor pixels. Further tests are under way to analyze these false positive samples as they were either present in the peri-tumoral regions, or appear having an inflammatory signature. Important features difficult to discern by conventional histopathology like tumor budding, tumor-stroma association, and inflammation, were easily identified by this methodology.
Conclusion: The discriminant infrared spectral wavenumbers enabled characterization of some of the malignancy associated biochemical alterations associated with mucin, nucleotides, carbohydrates and protein regions. This study constituting a label-free and non-destructive approach demonstrates the potential of IR spectral micro-imaging, combined with multivariate statistical image analysis, as a complementary tool to conventional histopathology for an automated and objective cancer diagnosis.

PO28

HOW TO SWITCH CONVENTIONAL GADOLINIUM CONTRAST AGENTS TO HYPERSENSITIVE DUAL-MODE PROBES FOR MRI

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Auteur présentant le résumé : Francoise CHUBURU

Résumé : Introduction: Gadolinium-based contrast agents (GdCAs) are widely used to enhance image contrast in MRI procedures. To obtain a good contrast, injection of high GdCA doses are required (0.5M). Recently, a correlation between the GdCA dechelation and the development of a new disease, Nephrogenic Systemic Fibrosis, was established.Consequently, EU and US health authorities have expressed some reservations for using and developing new GdCAs. In order to circumvent these limitations, we have developed GdCA-loaded polysaccharide-based nanohydrogels (GdCA-NPs).These nanohydrogels were elaborated in order to (i) ensure high Gd loadings in a highly hydrated nanostructure and (ii) be biocompatible and biodegradable.
Materials and methods: Nanohydrogels were synthesized using twoionotropic gelation procedureswith polysaccharides as biopolymers. MS325 (Vasovist®) and GdDOTP were the encapsulated GdCAs. Nanoparticle morphology was characterized by DLS/ELS and AFM. Nanoparticle Gd loading was evidenced by TEM-EDXS and quantified by ICP-OES. For each protocol, relaxivity studies were performed at 20 and 60 MHz (BrukerMinispec mq20 and mq60) for two intraparticular Gd concentrations and 1H NMRD profiles were measured at 37°C and 5°C between 0.01 to 300 MHz (Stelar Spinmaster FFC fast field cycling NMR relaxometer). MR imaging of nanoparticle phantoms was performed by using a 3.0 T MRI device (Achieva, Philips Medical Systems - Sense head 8 coil).
Results: GdCA-NPs with average hydrodynamic diameters between 100 and 550 nm were synthesized. High relaxities per Gd center were obtained or GdCA-NPs (r1 ~ 104 mM-1 s-1 and r2 ~ 195 mM-1 s-1 for MS325 and r1 ~ 65 mM-1 s-1 and r2 ~ 152 mM-1 s-1 for GdDOTP, at 60 MHz and 37°C). The NMRD profiles confirmed that the hydrogel structure of GdCA-NPs greatly amplified the magnetic properties of the encapsulated GdCAs. At 3T, T1- and T2-weighted images recorded forphantoms appeared significantly brighter, for lower Gd doses.
Conclusion: Incorporation of Gd chelates inside nanohydrogel matrices induced Gd relaxation enhancement which was clearly translated into a magnified contrast enhancement capability at 3T. Furthermore, these Gd nanohydrogels exhibited a powerful dual mode imaging.

PO30

VIBRATIONAL SPECTRAL IMAGING OF SINGLE CELLS GROWN ON TYPE I COLLAGEN SUBSTRATES

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Auteur présentant le résumé : Marie GUILBERT

Résumé : The cell microenvironment, especially extracellular matrix (ECM) proteins, play a key role in tumor progression process and cell response to anticancer drugs. Most in vitro studies have investigated the migratory and invasive properties of tumor cells in conventional cell culture systems such as plastic substrates. However, these systems do not take into account the microenvironment in which cancer cells develop in vivo complex interactions with their counterparts and with ECM proteins. Among these matrix proteins, type I collagen represents the major component in the body connective tissues, through which tumor cells usually migrate to form metastases, and which can be used as a preintravasation microenvironment.
In this study, we have investigated cell/matrix interactions by a multimodal approach using vibrational imaging, in order to characterize spectroscopic markers at tumor cells invasion front. To do so, HT1080 human fibrosarcoma cells, known to be highly invasive, were grown on 2D coatings of type I collagen. In a first step, spectral imaging of single cells was performed using infrared (IR) microspectroscopy in the transmission mode, with both conventional and Synchrotron sources; the latter allowing improving the spatial resolution. In a second step, HT1080 cells grown on type I collagen were analyzed by Raman imaging, using a 785 nm excitation. Spectral images obtained were analyzed by chemometric methods based on clustering algorithms, to extract spectral features of cell/collagen I interaction areas. Results showed a good discrimination between DNA/RNA, protein signals from cell and protein from matrix substrate, allowing highlighting spectroscopic markers specific to the tumor cell invasion front. This work demonstrates the potential of vibrational microspectroscopies for studying invasive processes of cancer cells in contact with their microenvironment, by rapid, sensible, non destructive and label-free imaging methodologies. Use of a synchrotron source allows acquiring more precise information, at the single cell level, on the cell/collagen contact areas.

PO31

HIGHLIGHTING INTRATUMORAL HETEROGENEITY AND PERITUMORAL AREAS IN HUMAN MELANOMA BIOPSIES BY INFRARED SPECTRAL MICROIMAGING

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Résumé : Scientific background: Infrared (IR) spectral microimaging is an efficient label-free optical method to probe the intrinsic biochemical composition of biological samples. Recent studies have shown its potential to detect and characterize cancerous tissues in their early stages, independently and without any morphological information, but only based on the molecular information of the sample. IR microimaging appears more informative than conventional histology and could thus be developed as a sensitive, non-destructive and objective diagnostic tool in clinical oncology. The discrimination between tumoral and surrounding tissues relies on highlighting subtle IR spectral differences. To this end, the development of an automatic and meaningful method for IR spectral data analysis is required.
Material and Methods: IR spectral images have been directly acquired on 10 µm thick formalin-fixed paraffin-embedded tissue sections of human cutaneous melanomas with different degrees of invasiveness (n=10 patients). An innovative Fuzzy C-Means (FCM) clustering based algorithm was developed in order to introduce the notion of nuance into the cluster membership of IR image pixels, and to automatically determine the optimal clustering parameters, i.e. the number of clusters and the fuzziness parameter. The user thus feeds the algorithm with infrared image, and gets back the clustering results, without managing any parameter. When applied on infrared images acquired on melanoma sections, the algorithm estimates color-coded clustering images which represent a real spectral histology of these samples, allowing to recover their different histological structures. Particularly, the tumoral and normal areas can be precisely localized.
Results: More than reproducing classical histology, our algorithm highlights additional information about the sample tissular structures. The notion of nuance introduced by FCM is valuable for the pixels located at the interface between tumoral tissue and its microenvironment. Heterogeneous transitional areas between tumor and marginal normal tissue are identified, what is not possible on hematoxylin-eosin stained section or by conventional clustering. In addition, this automatic approach of IR data processing permits to highlight the interconnectivity between heterogeneous intratumoral areas and between tumoral and peritumoral structures.
Conclusion: IR spectral microimaging associated with FCM clustering can be directly performed on paraffin-embedded tissue sections of human cancers. The results obtained in our preliminary study show significant potential for probing tumor progression and for early determination of tumor aggressiveness in cutaneous melanomas.

PO36

MONITORING OF THE INFLUENCE OF LRP-1 SILENCING ON A MALIGNANT CELL LINE AT THE MECHANICAL LEVEL USING ATOMIC FORCE MICROSCOPY

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Auteur présentant le résumé : Anthony LE CIGNE

Résumé : The scavenger receptor low-density lipoprotein receptor-related protein 1 (LRP-1) mediates the clearance of a variety of biological molecules from the pericellular environment, including proteinases which degrade the extracellular matrix in cancer progression. As such, LRP-1-dependant endocytosis has been widely studied as a potential target against cell invasion, and was found to have prognostic value. In addition to this role, LRP-1 is known to regulate the cell-to-matrix adhesion-deadhesion balance through an influence on the composition and turnover of attachment sites. This dual role leads to a variety of cellular responses regulating cellular migration under various physiopathological conditions, but how the latter role could affect the widely acknowledged anticancer potential of LRP-1 remains unclear. Recent data have provided evidence that cell migration is decreased by LRP-1 silencing, despite a verified increase in pericellular proteolytic activities. Here, we monitored the effects of LRP-1 silencing by a short hairpin-RNA (shRNA) strategy in a human thyroid carcinoma cell line at both the multiple-cell and single-cell levels, using atomic force microscopy (AFM). Force spectroscopy experiments in physiological conditions have shown that the profound morphological modifications that have been observed optically and by AFM after LRP-1 silencing are correlated with a significant increase in Young's modulus, from 5.26 kPa to 26.24 kPa, when a low-stiffness phenotype is normally observed for cancer cells with greater migration capabilities. Furthermore, transient transfection of cells with a vector expressing β1 integrin subunit fused with DsRed allowed us to carry out elastic modulus measurements by AFM at sites where integrin clustering takes place, which could be visualized directly by epifluorescence microscopy. We showed that LRP-1 silencing has a direct effect on integrin clustering dynamics, which can again be reflected by mechanical feedback at the individual cell level. Further investigations involving tip functionalization with antibodies directed against integrins subunits, and with specific substrat peptides, could allow us to extend our knowledge of the effect of LRP-1 silencing on mechanotransduction itself at the mechanical level.

PO5

MOLECULAR CHARACTERIZATION OF TIMP-1 BIOLOGICAL ACTIVITIES: AN ANTI-TUMORAL STRATEGY ?

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Auteur présentant le résumé : Laurie VERZEAUX

Résumé : Extracellular matrix (ECM) remodeling involves synthesis and degradation reactions. Proteolytic reactions are mainly catalyzed by Matrix Metalloproteinases (MMPs) which play a key role in pathological context such as tumor progression. MMPs activities are regulated by their natural inhibitors, the Tissue Inhibitors of Metalloproteinases (TIMPs). In Human, 4 TIMPs have been characterized with specific inhibitory activities towards MMPs. Besides this property, TIMPs exhibit other biological MMP-independent activities able to promote differentiation, apoptosis and proliferation in various normal and cancer cells. Our studies focus on these effects and particularly those mediated by TIMP-1. We have demonstrated that TIMP-1, independently of its MMP inhibitory activity, promotes survival of the UT-7 erythroleukemic cell line. Others groups have shown that TIMP-1 exhibit similar effects in adherent cells (breast epithelial cells, mesenchymal stem cells…). These processes activate downstream signaling pathways (PI3Kinase/Akt or ERK) and are mediated by a membrane complex-receptor which seems to be tissue specific. Indeed, we have identified a complex-receptor composed of CD44 and pro-MMP9 in UT-7 cells whereas this receptor consists of CD 63 and integrin β1 in breast epithelial cells.
TIMP-1 regions and amino acids involved in MMP inhibitory activities are well characterized, but those responsible of MMP independent activities remain to be identified. Our project aims to characterize TIMP-1 regions or residues involved in the MMP-independent biological activities in order to improve its antitumoral potential.
First, new bioinformatic approach using numerical simulations and normal mode analysis was used. This approach allows to study large amplitude movements of the protein and has led to the identification of a "pincer movement". A hinge region has been identified and proposed to be responsible for the movement. Amino acids in this region have been mutated in order to disturb the movement and to explore their role in TIMP-1 biological activity. TIMP-1 mutants were generated, produced and purified by affinity chromatography. Their ability to inhibit MMPs (MMP-1, -2, -3 and -9), to induce cell survival in different cell lines and to bind their identified receptor were tested.
We expect to produce TIMP-1 mutants which have lost their pro-survival activity but with a maintained or increased MMP inhibitory activity. These mutants could help us to go deeper into TIMP-1 functions in normal and pathological conditions but also to study TIMP-1 movement or interaction with its receptor. These results could be interesting to design novel inhibitors and open new perspectives in cancer therapy.

PO12

THE HIGH THROUGHPUT CELL-BASED SCREENING FACILITY OF IGBMC, ILLKIRCH, FRANCE

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Auteur présentant le résumé : Anne MAGLOTT-ROTH

Résumé : Founded in 2005 by the Cancéropôle Grand Est (CGE) for consolidating the potential of the Alsacian research, this creation has been made possible by initial financial supports from local governments and from the CGE. Besides, the facility takes revenues from contributions of granted research projects. Located at the CEBGS building of IGBMC, the platform has a 100 sqm laboratory with two P2 laboratories equipped for high throughput mammalian cell culture. The platform's core team consists of four engineers and one technician, with strong experience in RNA interference, gene expression, mammalian cell culture and cellular biology. A transfected cell array is a biotechnological tool to speed up the characterization of the role of genes suspected to be responsible for a particular cellular phenotype. It is based on a high throughput screening combined with single cell high content phenotypic analysis. The platform objective is to identify genes and pathways implied in human diseases, with high therapeutic potential for treatment, using molecule transfection in cells (siRNA, …). High throughput cell transfection and phenotypic analysis are operated on a TECAN robotic station in Class II cabinet and on two automated high content imaging and analysis systems, respectively. A high speed data transfer network has been implemented to fulfil all user needs in term of data storage and access, distance image analysis. Because of its powerful potential in Drug Discovery and in the understanding of the biology of the cell functions, the development of this facility is well suited to give support to diverse research projects covering Cancer, Stem cell & Development, Virology fields. In 2012, the facility joined the RNAi Global Intitiative consortium and acquired human and mouse genome siRNA libraries. It also joined the Ingestem national infrastructure to develop novel cell models using stem cell differentiation technology.

PO22

RESPONSE OF GLIOMA INITIATING CELLS EXPOSED TO CETUXIMAB IN DIFFERENT CONDITIONS OF OXYGENATION

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Auteur présentant le résumé : Cédric BOURA

Résumé : Glioblastoma (GBM) is the most common and aggressive brain tumors. In recent years, glioma initiating cells (GICs) have been identified in GBM. This population of undifferentiated cells, called also glioma stem-like cells due to their stemness properties, is highly resistant to conventional treatments and involved in recurrence phenomena. GICs are preferentially localized in specific regions differently oxygenated such as hypoxic or peri-vascular regions wherein a close relationship exists between GICs and endothelial cells. Overactivation of the epidermal growth factor receptor (EGFR) signaling is a major feature of GBM. Despite the obvious interest to target EGFR, clinical trials evaluating specific EGFR inhibitors have led to disappointing results. The involvement of GICs in the failure of EGFR inhibitors remains to be elucidated. Thus, we evaluated the in vitro response of GICs to cetuximab, a monoclonal antibody targeting EGFR, under different oxygenation conditions (normoxia/hypoxia).
GICs were harvested from 2 human glioma models (TCG3 and TCG9), expressing EGFR differently. GICs were exposed to cetuximab (20 µg/mL) during 48h under moderate hypoxia (1.5 % O2) or normoxia (21%O2). Cell viability was evaluated by MTT assay and cell counting, and their capacity of self-renewal by clonogenic assay. We evaluated also the expression of CD133 (marker of undifferentiated cells) by western blotting. Finally, using endothelial cell tube formation assay and wound healing assay, we tested conditioned media of GICs to evaluate their in vitro angiogenic potential.
The results showed that cetuximab decreased GICs viability of approximately 40 % in normoxia, contrary to hypoxia. Indeed, for low oxygen concentrations, cetuximab was not able to counteract an increase of GICs proliferation even for GICs expressing highly EGFR. However, cetuximab induced a significant decrease of the self-renewal capacity of GICs as well as the CD133 expression, in particular in hypoxia.
The GICs-derived conditioned media induced in vitro angiogenesis, but cetuximab treatment did not interfere significantly with the formation of the endothelial network, even though the anti-EGFR treatment was able to strongly decrease the endothelial cell migration.
In conclusion, the sensitivity of GICs to cetuximab seems to be largely depending of oxygenation conditions but cetuximab can prevent efficiently the self-renewal of GICs in particular under hypoxia. Moreover, the use of cetuximab can also decrease endothelial cells migration, a major step of angiogenesis establishment. Nevertheless, future investigations in association with other targeted therapies could be envisaged in order to eradicate efficiently this specific cell population in particular under hypoxia.

PO32

OPTIMIZATION OF ILLUMINATION IN INTERSTITIAL PHOTODYNAMIC THERAPY FOR HIGH-GRADE BRAIN TUMORS

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Auteur présentant le résumé : Magali TOUSSAINT

Résumé : Photodynamic therapy (PDT) for brain tumors appears to be complementary to conventional treatments (Cancer Treat Rev, 2012). Many studies show the major role of the vascular effect in tumor eradication by PDT called VTP for Vascular Targeted PDT (Photochem Photobiol Sci, 2008 ; Int J Radiat Oncol Biol Phys, 2009 ; Pharm Res, 2010). To promote this vascular effect, a selective targeting of neuropilin-1, mainly over-expressed by tumor angiogenic vessels, was investigated using a photosensitizer coupled to a ligand peptide.
For interstitial PDT (iPDT) of brain tumors guided by real-time imaging, multifunctional nanoparticles consisting of a surface-localized tumor vasculature targeting neuropilin-1 and encapsulated PDT and imaging agents, have been developed (Nanomedicine, 2011; PLOSOne, 2012). The selected nanoparticles are favourable to a photosensitizer targeting strategy for iPDT combined with MRI (magnetic resonance imaging) (Theranostics, 2012).
Photophysical properties of these nanoparticles with or without peptide grafting revealed a preservation of the attractive photophysical characteristics of the photosensitizer.
In vivo optical properties characterization of the tumor tissue led us to assess the absorption and diffusion coefficients values μa 5,5‧2,9cm-1 and μs 36,4‧13,8cm-1, respectively without nanoparticles injection and after nanoparticles intra-venous injection μa = 0,9‧0,7cm-1 and μs = 63,2‧16,1cm-1. After intravenous injection of the multifunctional nanoparticles into rats with intracranial glioblastoma, we demonstrate a positive contrast enhancement of the tumor tissue by MRI, allowing the optimization of the optical fiber implantation.
The experimental values of μa and μs of the tumor tissue were introduced into a model of light propagation for animals with intracranial tumors treated by iPDT. This model of light distribution demonstrates that the absorption and diffusion coefficients influence strikingly photons propagation into the tumor volume and moreover we highlight the diffusion limit when using a cylindrical plane diffuser to treat tumor thickness greater than 2mm.

PO37

PHARMACOKINETIC MODELING OF NANOPARTICLES-BASED AGENTS INTO GLIOBLASTOMA FROM MRI IMAGING

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Auteur présentant le résumé : Jean-Baptiste TYLCZ

Résumé :
Introduction. Traditional anti-cancer therapies, such as radiotherapy or chemotherapy, use standard treatment protocols without taking into account variability between individuals or groups of population. That generally leads to a large uncontrolled inter-individual variability and a lack of predictability of therapeutic responses. Recent developments on multifunctional nano-systems have opened new perspectives for tumor control by proposing nano-actuators and nano-sensors able to administer and measure damage caused by a treatment at a subcellular level.
A direct consequence was the emergence of nanoparticle-based therapies such as the targeted photodynamic therapy (PDT). Photodynamic therapy is one of the noninvasive ways of treating malignant tumors. It relies on the selective uptake of a photosensitizing molecule in a tumor followed by exposure to the appropriate wavelength of light to trigger the therapeutic effect. The delivery control of the photo activating agent into the cancer cells is one of the major factors which directly affect the therapeutic efficiency of the PDT.
Purpose. This study addresses the question to measure and to analyze the ability of two tested nanoparticles types to enhance the Magnetic Resonance Imaging (MRI) performance. In order to improve the in vivo tumor uptake of such nanoparticles, a data-driven modeling approach, integrating image processing, design of experiments, compartmental modeling and parameter estimation was developed to directly estimate pharmacokinetic parameters from a time sequence of MRI. Methods and Materials. The two compared nanoparticles types were silica-based with gadolinium oxide core as MRI contrast agent and a chlorin as photosensitizer. The first type had peptides added as targeting ligands, which is assumed to increase the selectivity to tumor neovessels of the nanoparticles. The second type described no peptide and was defined as the control. Nanoparticles were both tested in vivo in rats which were xenografted with U87glioblastoma cells. MR images of the rats brain were taken at a constant time period during an hour after the injection of the nanoparticles. A segmentation process was performed in order to automatically compute the mean intensity of the pixels within the tumor (assumed to be proportional to the amount of nanoparticles).
Modeling method. We propose a parametric model (based on a two-compartment model) structure suited to the characterization of the nanoparticles pharmacokinetics in the brain tumor. Model parameters are estimated from continuous-time MRI time series data with the CONTSID Matlab toolbox and are finally used to compare the pharmacokinetic performances of the tested nanoparticles.Results and conclusions. Results obtained from real in vivo data have confirmed the relevance of this approach to compare the characteristics of two different nanoparticles. Such a solution would be particularly suited to rapid pharmacokinetic screening studies.

PO16

IMPACT OF TGF· ON MIRNA EXPRESSION IN EPSTEIN-BARR VIRUS (EBV) INFECTED CELL LINES

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Résumé : MicroRNAs (miRNAs) are a large class of small (22nt) non-coding RNAs that negatively regulate gene expression, and have been shown to be key regulators in a variety of processes including development, cell cycle and immunity. Epstein-Barr virus (EBV) is an oncogenic gammaherpesvirus, endemic in humans, that encodes for twenty-five miRNAs. It is associated with several well-recognized malignancies, such as Burkitt's lymphoma, and nasopharyngeal carcinoma. We performed small RNA deep-sequencing from the EBV-positive Burkitt's lymphoma cell lines Mutu-I, Sav-I and Kem-I, following both 2h and 24h of TGF· treatment to identify differentially expressed miRNAs. A markedly differential expression of cellular and viral miRNAs was observed between on one hand the Mutu and Kem cell lines and the Sav cell line on the other hand. This difference is most likely due to the expression of the oncogenic protein LMP1 by the Sav cell line. Interestingly, induction of the lytic cycle via TGF· or the expression of LMP1 could be responsible for the up-regulated expression levels of some EBV miRNA: BHRF1-2 and BART7 and the oncogenic cellular miRNAs mir-155 and mir-146a. These observations were confirmed by real time RT-PCR and northern blot analysis in all cell lines tested. In addition, we also noticed a dramatic difference in the absolute amount of these two cellular miRNAs between the three cell lines analyzed. The regulation of these miRNAs via LMP1 may thus represent a key event in the lymphomagenesis of EBV positive Burkitt's lymphoma.

PO48

EPSTEIN-BARR VIRUS AND BREAST CANCER

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Auteur présentant le résumé : Hayette Sénia BENSABER

Résumé : Summary: In western Algeria, breast cancer ranks 1st. It represents 30% of cancers in women. The etiology of prostate cancer remains unknown studies have also reported the presence of the genome of Epstein-Barr virus (EBV) in tumor cells of breast cancer primarily in infiltrating types. This suggests a potential role of EBV in tumor progression **[1, 2].**
Objective : To test different techniques to identify the effect of EBV in the development of mammary carcinomas. We tried to provide clarification as to the presence of serological markers involved in tumor breast cancer,
Methods: This study included twenty-four patients in western Algeria, January 2009 to June 2009 in gynecology (CHUO), the Pasteur Institute of Sidi férruj and Developmental Biology Laboratory and differentiation. The techniques used: histological frozen sections, the ELISA, Immunofluorescence and Western blot [3].
Results: The extreme ages are 28 and with an average of 73ans 45.83ans. the left breast is the seat of the ICC and found more dominant, the majority of sera explored show positive serological profiles: VCA IgG antibody, positive in 20/24 patients, IgA and IgG VCA EA at 2/24, antibodies EBNA19/24 and VCA IgM is negative in all patients.
Conclusion: Our results are consistent with those found in the literature and the association between EBV and the etiology of breast cancer remains unclear. [4]

Keywords: EBV. Breast carcinomas, tumor progression.

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