ABSTRACTS' BOOKLET

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CENTRE DES CONGRÈS PROUVÉ
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- Nanomedicine & radiobiology
- Therapeutic & diagnostic biomarkers
- Immunology, cell therapy, CAR-T cells
- Clinical applications of liquid biopsies : ctDNA, CTC...
- Innovative techniques and applications in liquid biopsy : nucleosomes, exosomes...

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ORAL COMMUNICATION

THURSDAY, NOVEMBER 3RD 2022

EVALUATING TENASCIN C EFFECT ON THE RESPONSE OF BREAST CANCER CELL LINES TO IRRADIATION IN 2D AND 3D CULTURE MODELS

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Breast cancer is causing high death toll despite earlier diagnosis and therapy [1]. Radiotherapy remains one of the cornerstones to improve the outcome of cancer patients. However, the efficiency of this treatment depends not only on tumor cells but also on tumor microenvironment; seeing that the molecules of the extracellular matrix like tenascin C (TNC) could support tumor growth and enhance tumor progression and metastasis [3].
In this study, we evaluated the impact of TNC on the response of breast cancer cells (NT193 and PyMT1099) towards irradiation (IR). TNC expression was downregulated in these cells by ShRNA lentiviral vector (ShC: control cells and ShTNC: knockdown cells). 2D and 3D cellular viability and proliferation were assessed at different IR doses (2, 6, and 10 Gy) by trypan blue and CellTiter-Glo3D® tests, respectively. Clonogenic cell survival assay and quantification of DNA damage and repair (foci □-H2AX and 53BP1) were performed to assess the radiosensitivity of these cell lines. The migration of NT193 and PyMT1099 cells was also determined at 2D and 3D levels.
A dose-dependent inhibition of NT193 and PyMT1099 viability was observed for both cell lines in 2D and 3D culture models where the viability decreased by 30% at 2 Gy and by 50% at 10 Gy, at day 3 post- IR ($p<0.05$). A differential effect of IR on cell viability was only noticed at 3D level. The results also showed that ShC cells were more radioresistant than ShTNC with a survival fraction at 2 Gy for NT193 ShC at 0.49 ± 0.02 versus 0.39 ± 0.02 for NT193 ShTNC ($p<0.05$). This may be due to a rapid DNA damage repair in ShC cells. The migration of ShTNC cells was slowed down upon IR when compared to ShC cells.

These results suggest that TNC had a radioprotective effect on breast cancer cells. In vivo studies are being conducted to confirm the observed impact in the context of tumor microenvironment

Notes:

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CORE-SHELL MESOPOROUS NANOCARRIERS FOR COMBINED PHOTOTHERMIA AND DRUG DELIVERY

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The chemical design of smart nanocarriers, providing in one nanoformulation combined anticancer therapies, still remains a challenge in the field of nanomedicine. Among nanomaterials, iron oxide-based core-shell nanostructures have been already studied for their intrinsic magnetic hyperthermia features that may be coupled with drug delivery. [1] However, despite the great interest today for photo-induced hyperthermia, very few studies investigated the potential of such nanocarriers to combine photothermia and drug delivery.
In this work, we have addressed for the first time, the use of a novel type of nanomaterial, iron oxide@ stellate mesoporous silica core-shell, denoted IO@ STMS NPs which can ensure a near-infra red (NIR) light-induced photothermal effect combined with drug release.[2] Mesoporous silica coatings are particularly appealing given their biocompatibility, high surface area, pore tunability and versatile chemistry.[3]

Keywords : Nanomaterials - Mesoporous silica- Iron oxide- Photothermia- Drug delivery

References : D. Mertz, O. Sandre, S. Bégin-Colin, Biochimica et Biophysica Acta (BBA) - General Subjects 2017, 1861, 1617., A. Adam, S. Harlepp, F. Ghilini, G. Cotin, B. Freis, J. Goetz, S. Bégin, M. Tasso, D. Mertz, Colloids and Surfaces A: Physicochemical and Engineering Aspects 2022, 640, 128407., F. Perton, S. Harlepp, G. Follain, K. Parkhomenko, J. G. Goetz, S. Bégin-Colin, D. Mertz, Journal of Colloid and Interface Science 2019, 542, 469.,

Notes:

RADIOSENSITIZATION WITH GADOLINIUM CHELATE-COATED GOLD NANOPARTICLES REDUCES GLIOBLASTOMA INVASIVENESSS

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For the last two decades, several studies have been focused on the radio-enhancer effect of metal-based nanoparticles and their application to treat radioresistant tumors for which dose escalation cannot be considered [1,2]. S. Roux's research group has developed ultrasmall gold nanoparticles coated by gadolinium chelates, named as Au@DTDTPA(Gd), for MRI-guided radiotherapy. In vivo, using tumor xenograft model, encouraging results have been reported concerning the possibility to follow up Au@DTDTPA-Gd by MRI and to enhance tumor radioresponse [3,4].
For further investigation, we aimed at exploiting the radiosensitizing potential of Au@DTDTPA(Gd) nanoparticles when combined with external X-ray irradiation (RT) to treat glioblastoma. Using complementary biological models, we explored biological effects on tumor invasiveness, as the main the area of tumor extension was considered. Conventional 3D invasion assays showed that the combined treatments including Au@DTDTPA(Gd) nanoparticles and/or RT (10 Gy single dose; 5 x 2 Gy or 2 x 5 Gy) was able to significantly reduce tumor invasion. Monitoring of U87-GFP tumor progression using organotypic cultures or intracerebral grafts confirmed the anti-invasive effect of Au@DTDTPA(Gd) on irradiated spheroids. Most importantly, the combination of Au@DTDTPA(Gd) with irradiation drastically reduced the number, the viability and the aggressiveness of tumor cells able to escape from U87 spheroids. Notably, the combined treatments significantly reduced the proportion of escaped cells with stem-like features, that could lead to recurrence.

Keywords : Gold nanoparticles, Radiotherapy, glioma, invasiveness
References : P. Retif et al., Theranostics 2015, 5, 1030, S. Pinel et al. Adv. Drug. Deliv. Rev. 2019, 138, 344, C. Alric et al., J. Am. Chem. Soc. 2008, 130, 5908., I. Miladi et al., Small 2014, 10, 1116.

Notes:

THURSDAY, NOVEMBER 3RD 2022

PHOTOTHERMAL EFFICIENCY OF INDOCYANINE GREEN J-AGGREGATES BY NANOFORMULATION WITH CALIX[4]ARENE

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Extension of photodynamic therapy that does not require oxygen to interact with cells, photothermal therapy (PTT) is based on increasing the temperature inside cancer cells. PTT is a promising approach in cancer therapy due to minimal invasiveness and its use in the near infrared (NIR) region allowing a deep tissue penetration.
Indocyanine green (ICG) is a fluorescent contrast agent, approved by the United States Food and Drug Administration (FDA), for angiography in ophthalmology, in sentinel lymph node biopsy and detection of metastasis. The good absorption properties of ICG make it also ideal for PTT applications. However, its efficacy is restricted by intrinsic limitations including rapid photodegradation, instability in solution correlated with rapid clearance from the body (2-4min in circulation). These limitations led to the use of nanosized ICG-J aggregates (ICG-J).1 ICG-J aggregates are characterized by a 105 nm redshifted absorption (from 785 nm to 890 nm), a severe diminution of fluorescence and a better photothermal efficiency. Unfortunately, ICG-J quickly disassemble in the presence of plasma proteins that limits its in vivo efficiency2.
In our study, we utilized amphiphilic tetracationic calix[4]arene (CX) to engineered ICG-J/CX nanoprecipitation (Figure). This strategy is based on the work carried out in 2016 by Yasuda et al. They highlighted an improvement in the stability and in optical properties of ICG.3
Our ICG-J/CX nanoprecipitation, which could be easily and quickly prepared, possess close to neutral charge and a size around 130 nm and demonstrated high photothermal capacities, a decrease of photobleaching and excellent stability in biological medium. Moreover, the photostability under irradiation was found to be higher than that observed for free ICG or ICG-J. In vitro, on monolayer human pharyngeal adenocarcinoma cells (FaDu), these properties have contributed to improving cellular uptake, absence of dark cytotoxicity, and considerable decrease in the ICG concentration required to reach high photothermal. After 3min of irradiation (@1W/cm²-808 nm), only 12 µg/mL of ICG-J/CX are necessary to obtain 50% cell death against 102 µg/mL and 191 µg/mL for ICG-J and free ICG, respectively.4
Thus, our nanoprecipitation demonstrates his potential to open up new avenues for attaining cancer treatment.

Keywords : photothermal therapy, indocyanine green, calix[4]arene, head and neck cancer
References : R. Liu, J. Tang; Y. Xu, Y. Zhou, Z. Dai, Nanotheranostics, 2017, 1, 430-439, C. Cheung, G. Ma, K, Karatasos, J. Seitsonen, J. Ruokolainen, CR. Koffi, H. Hassan, W. Al-Jamal, Nanotheranostics, 2020, 4, 91 -106. , T. Jin, A. Komatsuzaki, S. Tsuboi, Y. Imamura, Y. Muraoka, T. Sakata, H. Yasuda, Med. Chem. Commun, 7, M.Millard, Y. Bernhard, N. Canilho, S. Grandemange, S. Parant, M. Mourer, HP. Lassalle, A. Pasc. Nanoscale, 2022

Notes:

IMAGING OF 3D HEAD AND NECK CANCER SPHEROIDS BY QUANTUM DOTS

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Head and Neck cancers (HNSCC) management mainly relies on surgery, however the exact delineation of tumor during surgery is a real hurdle. Consequently, whole tumor resection and obtaining negative margins are challenging. However, surgical margin is a major prognostic factor for patients' outcome. Currently, margin investigation relies on tissue frozen section analysis, but this process can be time-consuming. Development of intraoperative tools like fluorescence imaging could be of great help for real-time surgery. In this context, use of fluorescent nanoparticles called Quantum Dots (QDs), with exceptional optical properties could be very attractive. Indeed, their high fluorescence emission associated with photostability makes them ideal candidates for real-time and long-term fluorescence imaging.

The objective of the present study was to evaluate the ability of Cadmium-based QDs, emitting fluorescence at 610 nm, and conjugated to the A20FMDV2 (A20) peptide which targets the □V□6 integrin, to label 3D spheroids of human tongue cancer cells HSC-3. Targeting properties of QDs were studied in HSC-3 spheroids and then in coculture with MRC-5 healthy fibroblasts to better reproduce the impact of tumor microenvironment. The conducted study has demonstrated a much better labeling and penetration of A20-QDs compared to unconjugated QDs in HSC-3 spheroids. Furthermore, QDs labeling was 2-fold higher in cocultured spheroids (HSC-3/MRC-5) compared to monoculture (HSC-3). However, additional analysis demonstrated that A20-QDs labeling was aspecific and not due to integrin targeting, as QDs conjugated with scramble peptide exhibited same results as A20-QDs. Thus, we turned to RGD-QDs to reduce peptide size from 20 to 3 amino acids and to reduce QDs zeta potential. Results obtained on monolayers were encouraging, demonstrating a specific targeting of HSC-3 cells, as both unconjugated QDs and scramble-QDs exhibited no labeling. In the future, experiments will be performed in HSC-3 and HSC-3/MRC-5 spheroids to investigates RGD-QDs potential in 3D models.

Keywords : Quantum Dots, Spheroids, Imaging, Nanoparticles

Notes:

THURSDAY, NOVEMBER 3RD 2022

PROTEIN ARGININE METHYLTRANSFERASE 2 (PRMT2) IS INVOLVED IN ACUTE MYELOID LEUKEMIA THROUGH ITS ROLE ON INFLAMMATION

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A direct link between chronic inflammation and development of Acute Myeloid Leukemia (AML) has been highlighted in the past few years, demonstrating an interconnection between marked inflammatory phenotype and aberrant myeloproliferation in AML patients. Treating AML patients displaying a higher inflammatory signature with anti-inflammatory molecules resulted in significant increase of overall survival. Protein Arginine Methyltransferases (PRMTs) are epigenetic factors known to regulate gene expression through the methylation of histone tails. It has been previously reported that PRMT1, -4 and -5 inhibition displays anti-proliferative effects on AML models. In this study we investigated the role of another member of this family, called PRMT2, in the development of AML through its regulatory roles in inflammatory pathways.

We first determined from an AML cohort (Leucegene project, IRIC, Montréal, Québec, Canada) that patients with lower levels of PRMT2 expression have a trend toward worse survival compared to those which harbor higher ones. In addition, a GSEA revealed that these PRMT2low patients display an enrichment of their pro-inflammatory pathways compared to PRMT2high patients. Therefore, we hypothesized that PRMT2 could be a key regulator of inflammatory processes in AML. We thus used a PRMT2 knockout mouse model (Prmt-2KO) and a PRMT2 knockout human AML cell line (HL-60) to validate our hypothesis.

Although we demonstrated no difference in the bone marrow progenitors or mature cell populations of Prmt2KO mice compared to control, we observed a significant difference when treating Bone-Marrow Derived Macrophages (BMDM) obtained from Prmt2KO mice with LPS compared to control cells. Indeed, Prmt2KO BMDMs are more sensitive to LPS stimulation and express higher levels of pro-inflammatory cytokines, supporting our previous findings for a role of PRMT2 in the negative regulation of inflammatory processes.

In the HL-60 AML cell line model, we showed that PRMT2KO cells exhibit higher levels of phosphorylated STAT3 protein compared to wild type cells after LPS stimulation. PRMT2 could therefore be directly or indirectly involved in the STAT3 signaling pathway by preventing its activation, thus explaining the role of PRMT2 in the LPS-stimulated inflammatory process. Expression and secretion of inflammatory markers such as IL-6 and Ferritin are examined. Identification of protein interaction partners of PRMT2 in PRMT2 overexpressed HL-60 cells after stimulation with LPS as well as RNA-seq analysis, are under investigation.

Keywords : PRMT2, arginine methylation, AML, inflammation

THERAPEUTIC INTEREST OF AN IRON-BASED COMPLEX TO IMPROVE TREATMENTS AGAINST PANCREATIC AND TRIPLE NEGATIVE BREAST CANCER.

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With 9.6 million people dying worldwide in 2018, cancer represents one of the leading causes of death, despite a better understanding of the mechanisms of carcinogenesis and improved therapeutic strategies. In this context, our research field is focused in triple-negative breast cancer (TNBC) and pancreatic cancer, both of which are particularly aggressive and lack therapeutic solutions.

Our current work is focused on highlighting the antitumor activity of new Iron-based organometallic complexes via their ability to bind DNA. The complex called AIM3, has demonstrated a sustained antiproliferative action on several cancer cell lines of which mammary triple negative MDA-MB-231 pancreatic T3M4 and BXPC-3 and on two non-cancerous cell lines, Raw 264.7 macrophages and MCF10A cells (mammary epithelial cells). Interestingly, in these non-cancerous cells the antiproliferative effect was reversible. Our results demonstrate that the antiproliferative effect of AIM3 leads to senescence in mammary MDA-MB-231 and to apoptosis in pancreatic BXPC-3 and T3M4 cells. Moreover, we have demonstrated, in MDA-MB-231 cell line, the ability of AIM3 to potentiate radiotherapy and chemotherapy. Indeed, treating cells with AIM3 prior to ionizing radiations or chemotherapy led to accelerating the onset of apoptosis. Moreover, it has been demonstrated that AIM3 is effective on T3M4 cells resistant to gemcitabine treatment.

Altogether these results are very promising in order to propose AIM3 as a new therapeutic agent even if its mechanism of action still has to be determined. Thus, a transcriptomic study was carried out on the MDA-MB-231 cell line treated with or without AIM3. Interestingly the results of this study highlighted the modulation of the transcript level of genes known to be regulated by iron homeostasis i.e PPFIA4 and NDRG1. These genes, upregulated in our study, are also increased in the two pancreatic cancer cell lines T3M4 and BXPC-3 treated with AIM3.

Furthermore, the addition of FeSO4 to the cell culture medium containing AIM3, reversed the antiproliferative and cytotoxic effect of the molecule. These results therefore suggest that AIM3 could be able to impact iron homeostasis, either directly or indirectly. Thus, this study still in progress allow us to propose AIM3 as a new therapeutic agent for the treatment of those particularly aggressive cancers.

Keywords : Pancreatic cancer , triple negative breast cancer, iron homeostasis, iron based complex , proliferation, apoptosis, senescence

References : Yin, Li, Jiang-Jie Duan, Xiu-Wu Bian, et Shi-Cang Yu. « Triple-Negative Breast Cancer Molecular Subtyping and Treatment Progress ». Breast Cancer Research: BCR 22, n? 1 (9 juin 2020): 61. https://doi.org/10.1186/s13058-020-01296-5., Neoptolemos, John P., Jörg Kleeff, Patrick Michl, Eithne Costello, William Greenhalf, et Daniel H. Palmer. « Therapeutic Developments in Pancreatic Cancer: Current and Future Perspectives ». Nature Reviews Gastroenterology & Hepatology 15, n? 6 (juin 2018, Halbrook, Christopher J., Corbin Pontious, Ilya Kovalenko, Laura Lapienyte, Stephan Dreyer, Ho-Joon Lee, Galloway Thurston, et al. « Macrophage-Released Pyrimidines Inhibit Gemcitabine Therapy in Pancreatic Cancer ». Cell Metabolism 29, n? 6 (4 juin 2019), Coombs, G S, A A Schmitt, C A Canning, A Alok, I C C Low, N Banerjee, S Kaur, et al. « Modulation of Wnt/?-catenin signaling and proliferation by a ferrous iron chelator with therapeutic efficacy in genetically engineered mouse models of cancer ». Oncogen

Notes:

THURSDAY, NOVEMBER 3RD 2022

ITGB2 ACTIVATES EGFR AND MEDIATES ERLOTINIB RESISTANCE IN SMALL CELL LUNG CANCER

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Lung cancer is the leading cause of cancer-related deaths worldwide. Small cell lung cancer (SCLC) is an extremely aggressive type of lung cancer. Median survival of SCLC patients is 6-12 months. Epidermal growth factor (EGF) signaling plays an important role in triggering SCLC. Furthermore, reports have shown that growth factor-dependent signals and alpha-, beta-integrin (ITGA, ITGB) heterodimer receptors functionally cooperate to integrate their signaling pathways. However, the precise role of integrins regulating EGF receptor (EGFR) activation in SCLC is unknown. In the present work we detected high ITGB2 and ITGA2 expression levels in SCLC by meta-analysis of RNA-sequencing data deposited at The Cancer Genome Atlas and qRT-PCR-based expression analysis of retrospectively collected human lung tissue samples. In addition, we showed that ITGB6 and ITGA2 were highly expressed in non-small cell lung cancer (NSCLC). Further, high ITGB2 levels in SCLC correlated with enhanced EGF signaling. Protein extracts analysis in SCLC and NSCLC cell lines revealed that ITGB2 interacts and activates EGFR in an ITGB6-dependent manner. Moreover, ITGB2 loss-of-function sensitizes SCLC cells to Erlotinib, an EGFR-inhibitor. We uncovered a mechanism of ITGB2-mediated EGFR activation in SCLC that explains EGFR-inhibitor resistance independently from EGFR mutations.

Keywords : SCLC, integrin, EGF signaling, EGFR, KRAS, extracellular vesicles

Notes:

ORAL COMMUNICATION

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IN VITRO MODEL SETUP FOR TUMOR-ASSOCIATED NEUTROPHILS STUDY IN VITRO: FOCUS ON OSTEOSARCOMA CONTEXT

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Osteosarcoma is a rare primitive bone cancer, with an incidence of 0.8/100,000 per year. Patient survival at five years has not changed over the last two decades, neither have treatments, which remain aggressive and trigger side effects; neo-adjuvant and adjuvant chemotherapy accompanying excisional surgery. So new therapeutic targets are now expected. In many cancers, there is a growing scientific interest in the tumor microenvironment and in Tumor Associated Neutrophils (TANs). These neutrophils may exhibit two different profiles: pro- or anti-cancer (N2 or N1 respectively). They can be identified by membrane markers (CD195 for anti-tumor N1, CD182hi for pro-tumor N2...), compared to circulating neutrophils (PMN) (1), and also thanks to their ratio of IL-8 / TNF-a secretion.
It has been demonstrated that neutrophils can be polarized in vitro with cytokine cocktails (2). However, the possibility of inducing such a polarization with the sole concomitant presence of osteosarcoma cells and neutrophils has not yet been investigated.

We developed a co-culture model of primary human neutrophils with human osteosarcomas cell lines or their culture supernatants. We analysed PMN surface markers by flow cytometry and their cytokine secretion by ELISA. Results were generated from 10 independent healthy blood donors for each experiment. The Kruskal-Wallis test was first applied on the "co-culture" and "supernatant" groups. If the null hypothesis H0 was rejected, a Wilcoxon-Mann-Whitney stratified exact test was performed. A value of $p<0.05$ was chosen as the significance threshold.

Our results show that osteosarcoma cell lines have the ability to induce in primary human neutrophils a N2-like phenotype with decreased CCR5 and TNF-□ expression, and increased CXCR2 and PD-L1 receptor expression. We also succeed in polarize neutrophils to a N1 profile with lower cytokine concentrations and culture times than recently described in the literature (2). We observed lineages-dependent results; KHOS and MNNG cell lines influenced N2-like polarization more strongly than MG-63, which in turn showed stronger overall effects than Saos-2. Of note, KHOS and MNNG cell lines are described by the ATCC for having a carcinogenic potential that Saos-2 and MG-63 do not have. One may suggest a link between the virulence of cancer cells and their ability to direct the neutrophil response in their favor. At this stage of our work, we can think that the polarization is at least partially due to one or more secreted mediators since the osteosarcoma cell lines culture supernatants alone recapitulate the effects observed in direct co-culture, even in a lesser extent. The nature of these mediators is currently being investigated with particular attention to TGF-□, which may be secreted or bound to the cancer cell membrane, thus providing a clue to the enhanced effect in direct coculture model. The presence of polarization co-factors cannot be excluded and more ambitious proteomic and transcriptomic studies could provide some answers.

Finally, this work highlights the potential of N2 polarization by osteosarcoma cell lines in an in vitro model and paves the way for the potential screening of new molecules in the treatment of osteosarcoma by targeting neutrophil polarization.

Keywords : Neutrophils; Osteosarcoma; Polarization; Co-culture; In vitro
References : (1) Bonavita O, Massara M, Bonecchi R. Chemokine regulation of neutrophil function in tumors. Cytokine Growth Factor Rev. Août 2016;30:81 6, (2) Ohms M, Möller S, Laskay T. An Attempt to Polarize Human Neutrophils Toward N1 and N2 Phenotypes in vitro. Front Immunol. 2020;11:532.

RADIOTHERAPY SCHEME EFFECT ON PD-L1 EXPRESSION FOR LOCALLY ADVANCED RECTAL CANCER

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Background.
In locally advanced rectal cancer, radiotherapy (RT) followed by surgery have improved locoregional control, but distant recurrences remain frequent. Although checkpoint inhibitors have demonstrated objective response in several cancers, the clinical benefit of PD-1/PD-L1 blockade remains uncertain in rectal cancer.

Methods.
We collected data from biopsies and surgical specimens in 74 patients. The main objective was to evaluate the impact of neoadjuvant RT and fractionation on PD-L1 expression. Secondary objectives were to study the relation between PD-L1 expression and tumor regression grade (TRG), progression-free survival (PFS), overall survival (OS), and CD8 TILs infiltration.

Results.
Median rates of cells expressing PD-L1 pre- and post-RT were 0.15 (range, 0-17) and 0.5 (range, 0-27.5), respectively (p = 0.0005). There was no effect of RT fractionation on PD-L1+ cell rates. We found no relation between CD8+ TILs infiltration and PD-L1 expression and no difference between high-PD-L1 or low-PD-L1 expression and TRG. High-to-high PD-L1 expression profile had none significant higher OS and PFS compared to all other groups (p = 0.06). Median OS and PFS were higher in biopsies with >0.08 PD-L1+ cells. High-to-high PD-L1 profile and ypT0-2 were significantly associated with higher OS and PFS. This study did not show the differential induction of PD-L1 expression according to fractionation.

Conclusion.
We demonstrated an increase in PD-L1 expression after RT, but we did not find a differential induction of PD-L1 expression according to fractionation. Patients with high-to-high PD-L1 expression had better outcomes, suggesting that patients with "hot tumors" are more likely to respond to treatment. Future work needs to evaluate whether adding anti-PD-1/PD-L1 antibodies to neoadjuvant RT in this setting can enhance anti-tumor immune responses and translate into clinical benefit.

Keywords:
PD-L1 expression; fractionation; neoadjuvant radiotherapy; rectal cancer.
Funding. Cancéropôle EST : Projet Emergence 2020

Notes:

FRIDAY, NOVEMBER 4TH 2022

HIGH-THROUGHPUT PRECLINICAL SCREENING OF CD123 SCFV TO DESIGN 3RD GENERATION OF CAR T-CELL IN THE TREATMENT OF BPDCN PATIENT

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BPDCN is an aggressive hemopathy characterized by constant overexpression of the CD123 antigen on 100% of blasts identifying CD123 as an antigenic target for the development of targeted therapies. We have developed an anti-CD123 CAR T-cell immunotherapy to redirect T lymphocyte (TL) expressing a chimeric antigen receptor (CAR). Although CAR T therapy is well described in the literature, its design is a complex process requiring optimizations to ensure (i) strong and stable CAR expression on the surface of TL and (ii) efficient and specific recognition of the target antigen. These optimizations are crucial to maintain long-term therapeutic effects and avoid side effects related to the on-target, off-tumor effect.

We have produced five 3rd generation CD123 CAR T-cell (CD28, 4-1BB, CD3□) composed to a single chain fragment variable (scFv) with a distinct affinity for CD123. After showing high transduction efficiency and high CAR expression at the donor T cell membrane, we evaluated the functionality of the top four CAR-T. We showed that these CAR-T have the ability to activate and eliminate BPDCN cells while sparing cells not expressing CD123. For healthy cells with low CD123 expression such as monocytes, hematopoietic stem cells (HSC) or endothelial cells, we show that CAR-T had mild activation and low toxicity. Despite a slight elimination of HSC, we showed that these cells were able to regenerate hematopoietic progenitors. We also showed using two cell models and a patient cell model in vivo in mice that CAR-T could expand in mice, reduce tumor uptake as well as increase overall survival of mice. Finally, based on the best CAR-T previously determined, we have shown that we can produce this CAR-T from both BPDCN patient cells at diagnosis and after relapse. Furthermore, we show a reduction in tumor volume and an increase in overall survival in an in vivo mouse model.

In this project, we show that the choice of scFv to produce a CAR-T targeting CD123 is an essential parameter in order to eliminate leukemic cells while sparing healthy cells expressing the antigen weaklier.

Notes:

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THE ROLE OF CIRCULATING TUMOR DNA IN PATIENTS WITH CENTRAL NERVOUS SYSTEM LYMPHOMA

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Introduction: Clinical outcomes for patients with central nervous system lymphoma (CNSL) are remarkably heterogeneous, yet identification of patients at high risk for treatment failure remains challenging with existing methods. In addition, diagnosis of CNSL requires invasive neurosurgical biopsies that carry procedural risks and often cannot be performed in frail or elderly patients. Circulating tumor DNA (ctDNA) has shown great potential as a noninvasive biomarker in systemic lymphomas. Yet, previous studies revealed low ctDNA detection rates in blood plasma of CNSL patients. In this study, we utilized ultrasensitive targeted high-throughput sequencing technologies to explore the role of ctDNA for disease classification, MRD detection, and early prediction of clinical outcomes in patients with CNSL.

Methods: We applied Cancer Personalized Profiling by Deep Sequencing (CAPP-Seq) and Phased Variant Enrichment and Detection Sequencing (PhasED-Seq, Kurtz et al, Nat Biotech 2021) to 85 tumor biopsies, 131 plasma samples, and 62 CSF specimens from 92 CNSL patients and 44 patients with other brain cancers or inflammatory cerebral diseases, targeting 794 distinct genetic regions. Concentrations of ctDNA were correlated with radiological measures of tumor burden and tested for associations with clinical outcomes at distinct clinical time points. We further developed a novel classifier to noninvasively distinguish CNS lymphomas from other CNS tumors based on their mutational landscapes in plasma and CSF, using supervised training of a machine learning approach from tumor whole genome sequencing data and own genotyping analyses, followed by its independent validation.

Results: We identified genetic aberrations in 100% of CNSL tumor biopsies (n=63), with a median of 262 mutations per patient. Pretreatment plasma ctDNA was detectable in 78% of plasma samples and in 100% of CSF specimens, with ctDNA concentrations ranging from 0.0004 – 5.94% allele frequency (AF, median: 0.01%) in plasma and 0.0049 – 50.47% AF (median: 0.62%) in CSF. Compared to ctDNA concentrations in patients with systemic diffuse large B-cell lymphoma (DLBCL, data from Kurtz et al., J Clin Oncol, 2018), plasma ctDNA levels in CNSL were in median more than 200-fold lower. We observed a significant correlation of ctDNA concentrations with total radiographic tumor volumes (TRTV) measured by MRI, but no association with clinical risk scores (i.e., MSKCC score) or concurrent steroid treatment. Assessment of ctDNA at pretreatment time points predicted progression-free survival (PFS) and overall survival (OS), both as continuous and binary variable. Notably, patients could be stratified into risk groups with particularly favorable or poor prognoses by combining ctDNA and TRTV as pretreatment biomarkers. Furthermore, ctDNA positivity during curative-intent induction therapy was significantly associated with clinical outcomes, both PFS and OS. Finally, we applied our novel machine learning classifier to 207 specimens from an independent validation cohort of CNSL and Non-CNSL patients. We observed high specificity (100%) and positive predictive value (100%) for noninvasive diagnosis of CNSL, with a sensitivity of 57% for CSF and 21% for plasma, suggesting that a significant subset of CNSL patients might be able to forego invasive surgical biopsies.

Conclusions: We demonstrate robust and ultrasensitive detection of ctDNA at various disease milestones in CNSL. Our findings suggest that ctDNA accurately mirrors tumor burden and serves as a valuable clinical biomarker for risk stratification, outcome prediction, and surgery-free lymphoma classification in CNSL. We foresee an important potential future role of ctDNA as a decision-making tool to guide treatment in patients with CNSL.

Notes:

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GENOMIC SIGNATURES IN CTDNA FROM PATIENT WITH ADVANCED BREAST CANCER TREATED WITH ANTI-AROMATASE AND CDK4/6 INHIBITORS: FIRST RESULTS OF THE CICLADES-CE STUDY

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Introduction: In 2020, breast cancer (BC) represented 11,7% of overall cancer diagnosis, accounting for over 2 million cases. BCs can be categorized according to their molecular entities. Hormone-dependent cancer cells are defined by the expression of hormonal receptors (estrogen or progesterone). These types of BCs are eligible to endocrine therapy including anti-aromatase inhibitors (AI) and/or anti-cyclin-dependent kinase 4/6 (CDK4/6) inhibitors. However, AI can select subclonal cancer cell population bearing mutations of ESR1, PIK3CA and/or AKT1 gene, that can generate AI resistance. The objective of CICLADES-CE study, ancillary to CICLADES trial is to monitor these mutations using ctDNA in patients with advanced or metastatic BC treated with AI and CDK4/6 inhibitors to identify genomic signatures that can be used during follow-up of clonal evolution.
Materiel, patients, and methods: Twenty Formalin-Fixed Paraffin-Embedded (FFPE) samples from female patients diagnosed with advanced BCs and treated with AI were qualified and selected. The tumor cell percentage was determined by a pathologist. After microdissection, DNA was extracted using the AllPrep® DNA/RNA FFPE kit (Qiagen). Extracted DNA was sequenced with the NextSeq 550® (Illumina) using Hybridization Capture-based Target Enrichment and a 516-gene panel. Single nucleotide variants, copy number variants and telomere length were detected. Data obtained were then analyzed to identify highly mutated genes and specific mutations in genes of interest.
Results: Among the 20 samples, only 19 were sequenced. Our results showed that several genes involved in the PI3-Kinase pathway were mutated across several samples. No mutations of AKT and ESR1 genes were found. Moreover three mutational signatures were identified across the 19 samples, corresponding to validated COSMIC (Catalogue of Somatic Mutations in Cancer) signatures 1, 5 and 30. Those signatures are associated with DNA damage repair impairment and deamination of 5-methylcytosines.
Conclusion: The results correlate with the origin of the samples and the type of fixation. The analysis of mutations provides enough information to create a new panel of genes specific to our cohort for ctDNA follow-up. This also creates a clear baseline mutational landscape for the analysis of the other samples of the CICLADES cohort.

Keywords : Breast cancer, genomic signatures, ctDNA, anti-aromatases, CDK4/6 inhibitors

Notes:

BLOOD AND URINE BASED LIQUID PROFILING OF TELOMERASE REVERSE TRANSCRIPTASE (TERT) C228T AND C250T PROMOTER MUTATIONS WITHIN CIRCULATING TUMOR DNA FOR MONITORING DISEASE PROGRESSION IN UROTHELIAL BLADDER CANCER PATIENTS

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Liquid biopsy based screening for mutations within oncogenes has delivered promising results in various cancer entities. In our study we took a closer look at upregulated telomerase activity in urothelial bladder cancer patients. We present an approach to screen for telomerase reverse transcriptase (TERT) promoter mutations using circulating tumor DNA (ctDNA) extracted from blood and urine obtained from a cohort of 88 patients at various timepoints. Read-out was performed using droplet digital polymerase chain reaction. In summary, we were able to successfully detect TERT mutations in both tested bodily fluids and propose ctDNA in urine as a valid and potentially useful marker to monitor disease progression in bladder cancer patients

Keywords : bladder cancer, telomerase, ddPCR

References : Bell RJA, Rube HT, Xavier-Magalhães A, Costa BM, Mancini A, Song JS, et al. Understanding TERT Promoter Mutations: A Common Path to Immortality. Mol Cancer Res 2016;14:315–23., Sung H, Ferlay J, Siegel RL, Laversanne M, Soerjomataram I, Jemal A, et al. Global Cancer Statistics 2020: GLOBOCAN Estimates of Incidence and Mortality Worldwide for 36 Cancers in 185 Countries. Ca Cancer J Clin 2021;71:209–49.

Notes:

FRIDAY, NOVEMBER 4TH 2022

PROFILING OF CIRCULATING TUMOR DNA FOR COMPREHENSIVE TUMOR GENOTYPING AND CHARACTERIZATION OF CLONAL HETEROGENEITY IN PATIENTS WITH ADVANCED ANAPLASTIC AND POORLY DIFFERENTIATED THYROID CARCINOMAS

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Abstract

Introduction: Patients with advanced anaplastic thyroid carcinomas (ATC) and poorly differentiated thyroid carcinomas (PDTC) are characterized by dismal prognosis despite various therapeutic options. Molecular factors underlying treatment resistance in ATC/PDTC are poorly understood, in part due to the limited availability of repetitive tumor samples. Circulating tumor DNA (ctDNA) from blood plasma has emerged as a promising biomarker for noninvasive profiling of tumor mutational landscapes and disease monitoring across cancers. In this study, we applied an ultrasensitive high-throughput sequencing (HTS) technology to assess the role of ctDNA for comprehensive tumor genotyping before initiation of treatment and to characterize clonal evolution and the emergence of resistance mechanisms at disease progression in patients with ATC/PDTC receiving Lenvatinib/Pembrolizumab therapy.

Methods: We applied the AVENIO sequencing technology, which covers 77 genes and 192 kb genomic space (ctDNA Expanded Panel, Roche), to 30 plasma samples collected before start of therapy and 8 plasma samples obtained at disease progression from 30 ATC/PDTC patients (21 ATC, 9 PDTC). All patients were treated with Lenvatinib and Pembrolizumab within the phase II clinical trial ATLEP (EUDRACT No: 2017-004570-34) conducted at the University Medical Center Freiburg, Germany. We assessed tumor mutational landscapes at both time points and explored genetic mechanisms of clonal heterogeneity and treatment resistance.

Results: We identified genetic variants in 97% (29/30) of pretreatment plasma specimens by ctDNA profiling, with a median of 3.7 aberrations per patient (range: 1-9). The most frequent variants were observed in TP53 (31%), TERT promoter (24%) and EGFR (24%) genes. Genes involved in the MAP kinase pathway including BRAF, KRAS, NRAS, and MAP2K1 were found to be affected in 41% of patients, with BRAF V600E mutations detected in 10% and RAS variants identified in 24% of patients. Of note, while BRAF V600E and aberrations in RAS genes were mutually exclusive, BRAF and RAS variants co-occurred with TP53 mutations in 21% of patients, as reported before (Wang JR et al., JCO PO, 2022). Other receptor tyrosine kinase pathway mutations were observed in EGFR (24%), PDGFRA/B (17%), PIK3CA/AKT (7%), and FGFR2/3 (7%) genes. In 7 out of 8 plasma samples (88%) obtained at disease progression, we detected at least one somatic variant. In total, 46% of these aberrations were shared between the diagnostic and progression sample and 54% were unique to one of the specimens. In 5 patients, we found emerging somatic mutations, potentially representing resistance mechanisms. In ATC patients, emerging variants were observed in FLT1, TP53, NF2, FBXW7, DDR2 AKT2, and CSF1R genes. In PDTC patients, novel aberrations evolved in FLT1, PDGFRB, SMO, and TSC1 genes.

Conclusions: Profiling of ctDNA using the AVENIO platform allows accurate characterization of tumor genotypes in ATC/PDTC patients, both before initiation of treatment and at disease progression. Our findings suggest that ctDNA could serve as a robust biomarker for evaluation of clonal heterogeneity and resistance mechanisms in ATC/PDTC, potentially guiding targeted treatment decisions.

Notes:

DETERMINATION OF HOMOLOGOUS RECOMBINATION DEFICIENCY USING LIQUID BIOPSY IN PATIENTS WITH OVARIAN CANCER: RESULTS OF THE BOVARY PILOT STUDY

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Background: Ovarian cancer is the 15th leading cause of cancer-death related worldwide. The use of platinum-based and paclitaxel chemotherapies were landmark breakthroughs in the treatment of ovarian cancer. Cells with defective BRCA proteins are deficient in the repair of double-stranded DNA breaks by homologous recombination (HR). The PARP pathway can mitigate this deficiency by detecting single DNA strand breaks and activating a number of effector proteins to initiate repair. The inhibition of PARP in the presence of the homologous recombination deficiency (HRD) leads to a process called “synthetic lethality” and results in cell death from major genetic disarray. It is also now acknowledged that PARP inhibitors can beneficiate to a larger group of ovarian tumors, especially those with a deleterious genomic alteration of genes involved in HRD (HRR) or with genomic instability. The aim of this pilot study is to establish the feasibility of the determination HRR-panel and genomic instability in patients with ovarian cancer using peripheral blood as surrogate to tissue biopsy.

Methods: 20 patients with stage III or IV ovarian cancer were included. For all patients both FFPE tissue and plasma at baseline were available. Genomic instability and HRR-panel were determined using both Myriad MyChoice CDx Plus and Sophia Genetics HRD tests. cfDNA were extracted from plasma samples and analyzed using NGS for both HRR gene-panel and shallow whole genome sequencing (sWGS).

Results: Among 20 samples, 19 samples were available for analysis. All SNVs and indels identified in FFPE tissue were also found in the blood samples (Se=100%, Sp=100%). HRD results were available with both Myriad and Sophia Genetics tests for 17 samples. Only one discrepant case was found (OPA=94.1%). HRD status was available only for the 3 out of 17 blood samples that had the highest cfDNA concentration.

Conclusion: Liquid biopsy can be used as a surrogate to tissue biopsy for the determination of HRR-gene status but further optimizations are required for the determination of the genomic instability score.

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THERAPEUTIC DRUG MONITORING AND CIRCULATING TUMOR DNA IN PATIENTS WITH BRAF-MUTATED METASTATIC CUTANEOUS MELANOMA TREATED WITH BRAF AND MEK INHIBITORS: INTERIM ANALYSIS OF THE OPTIMEL STUDY.

Albane L'Huillier [1], Julien Scala-Bertola [2], Allan Kolodziej [2], Pauline Gilson [3], Marie Husson [3], Marie Rouyer [3], Julie Dardare [3], Andréa Witz [3], Margaux Betz [3], Nicolas Gambier [2], Agnès Leroux [4], Priscillia Tosti [5], Denise Bechet [5], Jean-Louis Merlin [3], Lionnel Geoffrois [1], Alexandre Harlé [3].

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Background:
Dabrafenib and Trametinib are kinase inhibitors (KI) indicated as first-line therapy of BRAF-mutated advanced melanoma patients, but half of the patients will develop a resistance within 12 months. Many studies highlighted KI pharmacokinetic (PK) impact on toxicities. However, data about the relationship between PK and efficacy are sparse. Circulating tumor DNA (ctDNA) has been shown to be a prognostic factor for metastatic melanoma patients undergoing specific treatment, and allows a better follow-up of patients. Here, we underwent a prospective interventional study to determine steady state plasma drug concentration (Cssmin) and ctDNA impact on outcome in melanoma patients treated with Dabrafenib (anti BRAF KI) and Trametinib (anti MEK KI). This is an interim analysis on the 19 first patients included in the OPTIMEL study.

Methods:
19 patients with BRAF-mutated advanced melanomas (metastatic or that can't undergo surgery), were included. All patients were treated with Dabrafenib and Trametinib. Two blood tests were performed at days t0, t15, t30, t90, t180, t270 and t365 or at disease progression (tp): one for the drugs monitoring, and the other for ctDNA analysis. The relationship between two quantitative parameters was assessed by the Spearman correlation coefficient.

Results:
A significant correlation between Trametinib Cssmin and BRAF ctDNA variant allele frequency (VAF) at t0 (r= 0.68; p=0.03; N=11) was found. No correlation between Dabrafenib Cssmin and BRAF ctDNA VAF at t0 was found. Higher Trametinib concentrations as well as high ctDNA concentrations at baseline were both associated with a lower PFS.

Conclusion:
The interim analysis shows the interest of KI concentrations and cfDNA at baseline. The analysis of the 35 patients of the complete cohort as well as the iterative samples should bring more insights.

Notes:

ORAL COMMUNICATION

SATURDAY, NOVEMBER 5TH 2022

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ULTRA-SENSITIVE MONITORING OF LEUKEMIA PATIENTS USING SUPERRCA MUTATION DETECTION ASSAYS

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Rare tumor-specific mutations in patient samples serve as excellent markers to monitor the course of malignant disease and responses to therapy in clinical routine, and improved assay techniques are needed for broad adoption. We describe herein a highly sensitive and selective molecule amplification technology - superRCA assays - for rapid and highly specific detection of DNA sequence variants present at very low frequencies in DNA samples. Using a standard flow cytometer we demonstrate precise, ultra-sensitive detection of singlenucleotide mutant sequences from malignant cells against up to a 100,000-fold excess of DNA from normal cells in either bone marrow or peripheral blood, to follow the course of patients treated for acute myeloid leukemia (AML). We also demonstrate that sequence variants located in a high-GC region may be sensitively detected, and we illustrate the potential of the technology for early detection of disease recurrence as a basis for prompt change of therapy.

Notes:

"EPITRANSCRIPTOMICS": A PROMISING SOURCE OF CIRCULATING BIOMARKERS FOR PERSONALIZED MEDICINE

Amandine Amalric [1], Aurore Attina [2], Amandine Bastide [1], Jerome Vialaret [2], Florence Boissiere [3], Evelyn Crapez [3], Emmanuelle Samalin [4], Eric Rivals [5], Christophe Hirtz [2], Alexandre David [1],

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- Introduction & Objectives

Despite significant progress in targeted therapies, colorectal cancer (CRC) remains a major cause of mortality and morbidity worldwide. Indeed, CRC survival is highly dependent upon stage of disease at diagnosis: though early stage shows 70 to 90% 5-year survival, once the tumor spreads out to distant organs, 5-year survival plummets toward 10%. Identification of accurate biomarkers, through molecular profiling in healthy and cancer patient samples, could improve diagnosis and promote personalized medicine. While genetic and epigenetic alterations of DNA are currently exploited as cancer biomarkers, their robustness is limited by tumor heterogeneity. Further, defining a set of biomarkers instead of one would maximize the prediction performance. Recently, cancer-associated alteration of RNA marks has emerged as a promising source of diagnostic and prognostic biomarkers.

Epitranscriptomics is an emerging field that encompasses more than 150 chemical modifications in all types of RNA. This renewed interest for chemical modification of RNA was bolstered by recent progress in detection techniques such as high throughput sequencing and mass spectrometry. These modifications fine-tune gene expression and play a role in key cellular processes in both physiological and pathological contexts. Therefore, it comes at no surprise that a growing number of studies have connected variations of specific modified nucleoside levels in solid/liquid biopsies with cancer onset and progression (Amalric et al., 2022; Relier et al., 2022).

Our goal is to exploit multiplex targeted mass spectrometry in order to establish RNA modification patterns that could be used for early CRC diagnosis and patient stratification.

- Methods

A method has been developed and optimized to quantify RNA modifications from blood plasma samples:

(1) Extraction of circulating oligomeric RNA (cRNA) and free nucleosides (free-nuc) then, digestion of RNA into nucleotides and dephosphorylated into nucleosides (Institut de Génomique Fonctionnelle, Montpellier).

(2) Separation of the nucleosides by reverse phase ultra-performance liquid chromatography (LC) on a C18 column coupled to a Shimadzu 8060 triple-quadrupole LC mass spectrometer (MS) in multiple reactions monitoring (MRM) positive electrospray ionization (ESI) mode (Plateforme de Protéomique Clinique, Montpellier).

(3) Multivariate analysis of the epitranscriptome and identification of "epitranscriptomic signature" to stratify the patients by using machine learning approaches (Laboratoire d'Informatique, de Robotique et de Microélectronique de Montpellier).

As a proof-of-concept, we have analyzed blood samples from a cohort of 41 CRC patients (Institut de Cancérologie de Montpellier) at different stages of the disease (Adenoma and grades 0, I, II, III, IV) and 20 healthy donors (Etablissement Français du Sang) as controls.

- Results

Among the 35 RNA modifications implemented in our LC-MS/MS method, we successfully detected 12 modifications in circulating RNA and 20 modifications as free-nuc. Noteworthy, while some of them display significant variation level in CRC patient vs. healthy donors' samples, others do not fluctuate.

Looking at multiple nucleoside levels rather than just one at a time could increase sensitivity, specificity and robustness of diagnosis/prognosis. A computer pipeline has been set up in order to identify "epitranscriptomic signatures" and test their prediction efficiency via machine-learning approaches. Interestingly, the prediction by an ar-

tificial intelligence algorithm of the healthy / CRC status reaches an accuracy of 91.7% from cRNA data and an accuracy of 100% from free-nuc data (cross-validation estimation).

- Discussion & Conclusion

This study shows that nucleoside quantification by multiplex targeted MS approaches could help early diagnosis of CRC. Yet, the limiter number of patient samples did not permit to discriminate cancer grades or types (e.g. BRAF mutated). A bigger cohort will be soon analyzed to validate the use of our “epitranscriptomic signature” for early CRC detection as well as patient stratification.

In the coming years, cancer studies in the blossoming field of epitranscriptomics will definitively translate into opportunities for clinical applications such as disease diagnosis as well as clinical decision-making. Easy to perform, fast, cost-effective, sensitive and reproducible methods are needed to evaluate the diagnostic power of these potential biomarkers.

Keywords : Epitranscriptomics, liquid biopsy, diagnostic biomarker, colorectal cancer, mass spectrometry

References : Amalric, A. et al. (2022). Quantifying RNA modifications by mass spectrometry: a novel source of biomarkers in oncology. Crit Rev Clin Lab Sci 59, 1–18. https://doi.org/10.1080/10408363.2021.1958743., Relier, S. et al. (2022). Multivariate Analysis of RNA Chemistry Marks Uncovers Epitranscriptomics-Based Biomarker Signature for Adult Diffuse Glioma Diagnostics. Anal Chem https://doi.org/10.1021/acs.analchem.2c01526.

Notes:

BRIGHTSENS DIAGNOSTICS: NEW TECHNOLOGY FOR RNA DETECTION IN LIQUID BIOPSIES

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Ultrasensitive and quantitative detection of RNA in clinical samples is the key to molecular diagnostics of viral and cancer diseases. In order to detect the extremely low concentration of target RNAs, enzymatic amplification of the target RNA, such as Reverse Transcription Polymerase Chain Reaction (RT-PCR), is typically required. The latter is a complex multistep method, which needs dedicated equipment and experienced staff. BrightSens Diagnostics in collaboration with the University of Strasbourg develops a simple, rapid and cost-effective method for RNA detection based on fluorescent polymeric nanoparticles functionalized with DNA. The outstanding brightness of these NPs (equivalent to >1000 organic dyes) enables direct, amplification-free detection of target RNA at femtomolar concentrations, which corresponds to 25-30 RT-PCR cycles. The current challenges of this method are to further increase sensitivity to the RNA target and specificity to mutations. Moreover, we aim to expand the technology to both short and long nucleic acids sequences, which would enable detection of microRNA, long noncoding RNA and mRNA markers in oncology as well as RNA of viruses (e.g. SARS-Cov-2). Within the recently funded i-Lab project, we focus particularly on detection of microRNA cancer markers in liquid biopsies (blood). We are particularly interested in lung, breast, digestive and pancreatic cancers, which are among the most common in oncology. In a first step, we will propose kits for research use only and collaborate with research groups on the development of new protocols for molecular diagnostics in clinics. Ultimately, we aim to develop kits for point-of-care RNA detection in blood and saliva for rapid diagnostics, guide therapeutic choice (companion diagnostics) and continuous follow-up of patients.

Keywords : Diagnostic, innovation, liquid biospy, biomarker

Notes:

SATURDAY, NOVEMBER 5TH 2022

EPIGENETIC BIOMARKERS OF MELANOMA: BIOINFORMATICS ANALYSIS OF PUBLIC DATA AND PROOF OF CONCEPT

Hadjadj-Aoul A., Overs A., Caël B., Peixoto P., Guittaut M., Molimard C., Hervouet E., Selmani Z.

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Representing 4% of cancers, melanoma is a skin tumor that is a major public health problem because 50% of them are diagnosed at the metastatic stage. Moreover, 70% of these lesions are mutated (BRAF, NRAS mainly) and can therefore benefit from targeted therapies (anti-BRAF). Post-treatment monitoring of residual disease can be performed on circulating tumor DNA by digital PCR. This hypersensitive molecular biology technique can detect BRAF or NRAS mutations in peripheral blood. However today, there is no marker available for 30% of melanomas.

By in silico analysis of public databases (TCGA and GEO), we were able to identify 6 epigenetic markers specific to all types of melanoma, detectable in liquid biopsy. In a second step, we designed primers usable in digital PCR and allowing to target the CpG of interest for two markers : TLX3 and HOXD13.

In a local cohort of patients, these markers were then validated in vivo on tumor tissues (melanoma, n=28) and on non tumor tissues (healthy skin n=8 and naevi n=6). Hypermethylation of TLX3 and/or HOXD13 was observed in 78.6% of melanomas analyzed. And this hypermethylation in melanomas was significant specific compared to non-tumor tissues (TLX3 : p <10-3 ; HOXD13 : p <10-3).

Their detection in liquid biopsy and their use in personalized medicine, are major assets of these non-invasive biomarkers. Moreover by their specificity and sensitivity, these markers could be of interest in the screening, diagnosis, and follow-up of residual disease in all melanomas, regardless of their mutational status.

Notes:

POSTERS

IDENTIFICATION OF PREDICTIVE FACTORS FOR THE RESPONSE TO ANTI-PROGRAMMED CELL DEATH PROTEIN 1 (PD1) IMMUNOTHERAPY IN HEAD AND NECK SQUAMOUS CELL CARCINOMA (IPRICE)

Mickael Burgy [1]

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This project aims to organise the sampling of blood and tumor at key points of the standard of care of patients with recurrent or metastatic squamous-cell carcinoma of the head and neck (HNSCC). This will allow to identify new potential predictive biomarkers of efficacy of immunotherapy and to investigate the evolution of the tumoral microenvironment after successive systemic treatments.
Tumor and blood samples will be collected on patients treated by anti-PD1 immunotherapy at different timepoints. Tumor samples will be collected (i) before initiation of immunotherapy, (ii) at 50 days after initiation of immunotherapy and, optionally, in case of disease progression, (iii) before the initiation of the new line of chemotherapy and (iv) at 50 days after initiation of chemotherapy. Blood samples will be collected : (i) before initiation of immunotherapy, (ii) at each cycles of treatment until 84 days after initiation of immunotherapy and, optionally, in case of disease progression, (iii) before the initiation of the new line of chemotherapy and (iv) at each cycle until 50 days after initiation of chemotherapy (maximum 2 samples per month).

Keywords : Spatial transcriptomics, head and neck squamous cell carcinoma, microenvironment, immune repertoire, immunotherapy

Notes:

COMPARISON CF DNA AND CELLCOUNT OF 48H CEC IN EARLY AN LATE BREAST CANCER

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Introduction.
Maintrac (no enrichment , RBC lysis , fluorescent microscopic detection)counts CEC after 48h rest at room temperature .This timepoint differs with all other CEC detection methods. We interprete CEC biology as a proces driving cells in a hypoxic environment into an EMT, allowing to enter circulation, confronted with a this hyperoxic state a „reoxygenation shock „, leads to loss of many surface proteins blocking detection(1) In a further step platelets cover CEC and lead to complete invisibility (Pachmann K,personal communication). After 48 resting in a hypoxic situation platelets disappear and surface proteins reappear now allowing detection again.cf DNA are the remnants of died cells and are not involved in the abovementioned"CEC entering circulation events. Comparing amounts of cf DNA and CEC were sofar not equivocal (2). Methods. CEC were measured by flowmicroscopic detection (Amnis Flowsight) with a panel of antibodies including CD45,DAPI,EPCAM an DAA .Blood was collected in cfDNA collection tubes (Roche) cf DNA Extraction with Maxwell RSC,cfDNA measured with the QBit device .Results.We present first data comparing 48H CEC with cf DNA, 154 Patients after BC in aftercare and 45 Patients with advanced BC.cfDNA ranged from 0,5 to 29,9 ng/muL CEC48H from zeroto 34000 CEC pro ml.
A clear correlation between amounts of cf DNA and CEC numbers including living and dead EPCAM positive cells was found .In contrast to non 48 H measurements , cf DNA mirrors CEC counts.Sorting of 48H CEC should allow higher enrichment of ctDNA for genetic profiling.

Keywords : 48HCEC,cfDNA, Maintrac,Reoxygenation schock
References : , , Bartkowiak K, Koch C, Gärtner S, Andreas A, Gorges TM, Pantel K. In Vitro Modeling of Reoxygenation Effects on mRNA and Protein Levels in Hypoxic Tumor Cells upon Entry into the Bloodstream. Cells. 2020 , Dawson SJ, Tsui DW, Murtaza M, Biggs H, Rueda OM, Chin SF, Dunning MJ, Gale D, Forshew T, Mahler-Araujo B, Rajan S, Humphray S, Becq J, Halsall D, Wallis M, Bentley D, Caldas C, Rosenfeld N. Analysis of circulating tumor DNA to monitor metastatic breast cancer.

Notes:

ONGOING LIQUID BIOPSY-BASED ANCILLARY STUDIES AND CLINICAL TRIALS AT INSTITUT DE CANCÉROLOGIE DE LORRAINE

Jean-Louis Merlin [1]

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The interest of the detection of cell-free DNA (cfDNA) in plasma is now demonstrated for the early- detection, monitoring and theranostic in cancer. Several clinical trials involving different assays for the detection of cfDNA in patients with different cancer localization are currently ongoing at Institut de Cancérologie de Lorraine.

- Melanoma: OPTIMEL trial is an observational study aiming at evaluating the relationship between ctDNA and kinase inhibitors plasma concentrations in patients with metastatic melanoma, treated with anti-BRAF and anti-MEK therapy. 35 patients have been prospectively included. The interim analysis is reported (see Lhuiller A et al abstract).
- Ovarian cancer: BOVARY-Pilot study is aiming at establishing the feasibility of the determination HRR-panel and genomic instability in patients with ovarian cancer using peripheral blood as surrogate to tissue biopsy. The first results are reported (see Harle A et al abstract). BOVARY CE trial is in progress and will enlarge the study aiming at the evaluation of clonal heterogeneity and clonal evolution in newly diagnosed patients with high grade adenocarcinoma of ovarian origin.
- Breast cancer: CICLADES CE is an ancillary study to the CICLADES trial consisting in monitoring of ctDNA mutations during real-life follow-up of patients with metastatic breast cancer treated with endocrine therapy. CICLADES CE explores the tumor clonal evolution using extensive NGS and follow up of selected mutations over time.
- Bladder cancer: URODX-FGFR3 trial consists in evaluating potency of urinary analysis combining VisioCyt® automatized cytology and FGFR3 mutations analysis for the detection of bladder cancers.
- Pancreas cancer: TransPANDAS is an ancillary study to PANDAS (Prodige 44) two arm, prospective, multicenter randomized phase II trial of neoadjuvant modified Folfirinox regimen, with or without preoperative concomitant chemoradiotherapy in patients with borderline resectable pancreatic carcinoma. This study is based on the analysis of cfDNA for the detection with next generation sequencing of somatic mutations and circulating miR analysis. End of inclusions in this trial is planned at Q4 2022.
- Peritoneal carcinomatosis: PIPADN is a pilot study evaluating the relationship between ctDNA concentration and the evolution of peritoneal carcinomatosis under Pressurized Intra Peritoneal Aerosol Chemotherapy (PIPAC).
- Melanoma: PERCIMEL multicentric trial (PERsonalized follow-up of ctDNA in patients with resected stage III and IV melanomas) trial is aiming at evaluating the predictive value of postoperative ctDNA for disease-free survival in patients receiving anti-BRAF/anti-MEK targeted therapy or immunotherapy.

Notes:

DEVELOPMENT OF A MODEL TO STUDY THE DIAGNOSTIC PERFORMANCE OF FLUORO-ESTRADIOL RADIOTRACER IN PET IMAGING IN PRECLINICAL MODELS OF MUTATED ER BREAST CANCER

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Breast cancer (BC) is the most common cancer in the world and the deadliest in women in France (1).BCs are currently classified into five intrinsic subtypes, based on the expression of 3 receptors: the EGF type 2 receptor (HER2), the progesterone receptor (PR) and the estrogen receptor (ER). The ER+ subtype accounts for 70% of breast cancers and is associated with the best 5 years overall survival (2). However, in case of relapse, this time frame drops (9 months to 3 years) (1), and tumor cells usually develop constitutive activation mutations of the ER alpha (10-50% of cases) (3). To date, 62 mutations of the receptor have been identified and are mostly localized in the ligand-binding domain (4).Diagnosis of this pathology is based on imaging exams such as Positron Emission Tomography (PET) with 18FDG (18Fluorodeoxyglucose), a glucose analogue. However, it does not allow to precise ER status of the tumors. In order to answer this question, a new radiotracer, an estradiol analog that can bind to the ER, has been developed: 18FES (18Fluoroestradiol). But the ability of this radiotracer to bind to the different mutated forms of ER has not yet been determined.

The aim of this work is to specify the affinity of this radiotracer for various ER mutants in order to precise the ER status of breast tumors during diagnosis (primary site and metastasis) and thus propose the best therapeutic strategy.

We have developed an ER inducible Tet-ON breast cancer cell model. Six clones corresponding to the most frequent mutations found in patients at the metastatic stage (Y537S/C/N, D538G, E380Q and L536H) (4) were generated. In vitro assays were performed to validate the inducibility of the system. Finally, the functionality of the system has been validated in vivo by injecting cancer cells orthotopically into SCID mice and realizing IHC experiments.
MicroPET will be soon carried out in order to estimate the effect of each mutation on the 18FES binding but also to compare this PET SUV (standardized uptake value) with thus obtained with the 18FDG. Subsequently, we will specify the affinity constants of FES for the different mutants using micro-scale thermophoresis (MST).

All these findings should allow to valorize the use of 18FES in PET imaging for the diagnosis and the patient follow-up for ER+ breast cancers.

Keywords : Breast cancer, PET imaging, Estrogen receptor mutations
References : Donald Courtney et al. 2022 Irish Journal of Medical Science , P.E. Goss et al. 2016 The New England Journal of Medicine , Weiyi Toy et al. 2013 Nat Genet , Derek Dustin et al. 2019 Cancer

Notes:

MIR-30A-3P AND MIR-30E-3P INFLUENCE TUMOUR PHENOTYPE OF HEAD AND NECK SQUAMOUS CELL CARCINOMA BY TARGETING TGF-BETA/BMP SIGNALLING.

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Head and Neck Squamous Cell Carcinoma (HNSCC) is the 6th most common cancer worldwide (Globocan, 2020). 70% of patients exhibit advanced HNSCC stage at diagnosis and their 5-year survival rate is less than 50% because therapeutic management is only based on the TNM classification and no reliable biomarkers for diagnosis or prognosis are available. In the last few years, miRNAs profile was studied in HNSCC and differences of expression between tumour vs healthy tissue were highlighted. Studies have suggested that miRNAs have the potential to become biomarkers (Sethi et al., 2014). Here, we studied members of the miR-30 family which act as antitumor miRNAs in HNSCC. We investigated the role of miR-30a-3p and miR-30e-3p expression on HNSCC relapses and clinical prognosis. Their expressions were analysed in 122 HNSCC HPV-negative, locally advanced tumours. miRNAs level of expression was correlated with clinical data and it showed that low level of miR-30a-3p and miR-30e-3p was correlated with higher risk of relapses and lower survival rate. Thus, these miRNAs have the potential to become biomarkers for relapses and prognosis. Next, we studied in vitro features of relapses and survival by overexpressing miR-30a-3p and miR-30e-p in HNSCC cell lines. Colony formation, proliferation and apoptosis assays showed that survival was reduced when miRNAs were overexpressed in cells. In addition, spheroid evasion assay showed that migration was also reduced compared to control. Then, we wanted to determine the underlying pathway by which miRNAs affected these malignant biological functions. Screening TCGA database and evaluating connection of identified targets using STRING showed that miR-30a-3p and miR-30e-3p target several genes that happen to belong to the TGF-□ network. Repression of this pathway was confirmed using RT-qPCR, western blot and immunofluorescence analyses. Pharmacological inhibition of the most affected targets, TGFBR1 and BMPR2, recapitulated effects observed by direct expression of miRNAs, suggesting that miRNAs exert antitumor activity through the inhibition of TGFBR1 and BMPR2. Beside their potential as biomarkers, miRNAs might be used to target one of the most important immunosuppressive pathway in HNSCC.

Keywords :

References : Sung, H., Ferlay, J., Siegel, R., Laversanne, M., Soerjomataram, I., Jemal, A. and Bray, F., 2021. Global Cancer Statistics 2020: GLOBOCAN Estimates of Incidence and Mortality Worldwide for 36 Cancers in 185 Countries. CA: A Cancer Journal for Clinicians,, Sethi, N., Wright, A., Wood, H. and Rabbitts, P., 2014. MicroRNAs and head and neck cancer: Reviewing the first decade of research. European Journal of Cancer, 50(15), pp.2619-2635.

Notes:

DETECTION OF RNA BIOMARKERS IN BIOFLUIDS BY ULTRABRIGHT FLUORESCENT NANOPARTICLES

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Nucleic acids are important biomarkers in cancer and viral diseases, however their rapid detection may be difficult due to their presence at very low concentration. So far, the gold standard of RNA diagnostics remains RT-PCR due to its high sensitivity achieved by target amplification. However, this method is generally used for samples after dedicated RNA extraction and overall, the method is rather expensive and requires experienced staff. Previously, we described a new method for the detection of nucleic acids in solution based on ultrabright dye-loaded polymeric nanoparticles (NPs), functionalized with nucleic acids. It enabled amplified detection of DNA/RNA in solutions with picomolar limit of detection, and on surfaces with single-molecule sensitivity, compatible with smartphone-based sensing. Also, these DNA-functionalized NPs have already been validated for detection of microRNA in cell extracts.
In the present work, we aimed to develop an improved method based on DNA-NPs for detection of target RNA biomarkers directly in biological fluids without RNA extraction. It requires rather simple protocol of sample preparation followed by an automated detection with a plate reader. Our method was able to detect RNA molecules of 40 to 1000 nt length in complex medium (RNA extracts and nasopharyngeal swabs) with limit of detection in the femtomolar concentration range. Our next challenge ahead is the detection of cancer-related biomarkers in biofluids like liquid biopsies (serum or plasma), saliva and urine.

Keywords : fluorescent polymer nanoparticles, RNA biomarker, clinical diagnostics
References : Melnychuk, N. & Klymchenko, A. S. DNA-Functionalized Dye-Loaded Polymeric Nanoparticles: Ultrabright FRET Platform for Amplified Detection of Nucleic Acids. J. Am. Chem. Soc. 140, 10856–10865 (2018)., Melnychuk, N., Egloff, S., Runser, A., Reisch, A. & Klymchenko, A. S. Light-Harvesting Nanoparticle Probes for FRET-Based Detection of Oligonucleotides with Single-Molecule Sensitivity. Angewandte Chemie International Edition 59, 6811–6818 (2020)., Severi, C., Melnychuk, N. & Klymchenko, A. S. Smartphone-assisted detection of nucleic acids by light-harvesting FRET-based nanoprobe. Biosensors and Bioelectronics 168, 112515 (2020)., Egloff, S., Melnychuk, N., Reisch, A., Martin, S. & Klymchenko, A. S. Enzyme-free amplified detection of cellular microRNA by light-harvesting fluorescent nanoparticle probes. Biosens Bioelectron 179, 113084 (2021).

Notes:

DDB2 IMPACTS EPITHELIAL-TO-MESENCHYMAL TRANSITION AND IMPROVES SENSITIVITY TO CHEMOTHERAPY OF PANCREATIC DUCTAL ADENOCARCINOMA CELLS

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Background: Damage specific DNA binding protein 2 (DDB2) is originally implied in the recognition of ultraviolet-induced DNA damage and the initiation of nucleotide excision repair (NER) process. This protein also demonstrated dual roles in several cancers, acting either as an oncogene or a tumor suppressor gene depending on cancer localization. In this study, we investigated the unresolved role of DDB2 in pancreatic ductal adenocarcinoma (PDAC).
Methods: The expression level of DDB2 in pancreatic cancer tissues and its correlation with patient survival were first evaluated using publicly available data. Second, two PDAC human cell models with CRISPR-modified DDB2 expression were developed: DDB2 was repressed in native DDB2-high T3M4 cells (T3M4 DDB2-low) and DDB2 was overexpressed in native DDB2-low Capan-2 cells (Capan-2 DDB2-high). Immunofluorescence and qPCR assays were used to investigate epithelial-to-mesenchymal transition (EMT) in these models. Migration and invasion properties of the cells were also determined using wound healing and transwell assays. Sensitivity to 5-fluorouracil (5-FU), oxaliplatin, irinotecan and gemcitabine were finally investigated using crystal violet assays.
Results: DDB2 expression level was reduced in PDAC tissues compared to healthy pancreatic tissues and DDB2-low levels were correlated to shorter disease-free survival in PDAC patients. The repression of DDB2 in T3M4 DDB2-low cells enhanced the transcription of SNAIL, ZEB1 and TWIST (EMT transcription factors), increased the expression of the N-cadherin (mesenchymal marker) and decreased the levels of the E-cadherin (epithelial marker). Conversely, DDB2 overexpression in Capan-2 DDB2-high cells increased E-cadherin levels and decreased N-cadherin levels. The migration and invasion properties were negatively correlated with DDB2 expression level in both cell models. DDB2 has no effect on T3M4 cell sensitivity to chemotherapy but sensitized Capan-2 cells to 5-FU, oxaliplatin and gemcitabine.
Conclusion: Our study highlights the potential tumor suppressive effects of DDB2 on PDAC progression. DDB2 could thus represent a promising therapeutic target and a prognosis and predictive biomarker in patients with PDAC.

Keywords : Pancreatic cancer, epithelial-to-mesenchymal transition, DDB2, biomarker

Notes:

EFFECT OF IN VITRO STARVATION ON THE RESPONSE OF BREAST CANCER CELLS TO CHEMOTHERAPY

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Triple-negative breast cancer (TNBC) still remains a major challenge in oncology. Indeed, the benchmark treatment is conventional chemotherapy but with limits due to treatment resistance and relapse. Therapeutic fasting, mimicked by in vitro starvation (IVS) characterized by a decrease in serum and glucose levels, could potentiate the response of breast cancer cells to chemotherapy (Raffaghello et al., 2008; Lee et al., 2012). Our objective was to characterize the response of TNBC cells cultured in 2D or in 3D conditions to IVS and to determine its influence on chemotherapy sensitivity.

The experiments were performed on Hs578T TNBC cells. DMEM was supplemented either with 10% FBS and 4.5 g/L glucose (control condition) or with 2.5% FBS and 0.5 g/L glucose (IVS condition). In 2D and 3D cultures (overlay method), cells were grown during 24 h in IVS and treated for 24, 48 and 72 hours with doxorubicin or FEC (5-fluorouracil, epirubicin, cyclophosphamide).

In 2D culture, Hs578T cell numbers were lower in IVS than in control condition. This was consistent with an increase in the cell number in the sub-G1 compartment of the cell cycle as well as an increase in annexin V positive cells. However, IVS never potentiated the effects of doxorubicin or FEC. In 3D culture, IVS condition did not change mammospheres viability (Phosphatase acid assay). It did not improve doxorubicin effects but potentiated FEC efficiency. Overall, our study suggests that IVS could potentiate chimiotherapy only in 3D models. The metabolic changes induced by IVS are currently analyzed in TNBC mammospheres. A transcriptomic analysis was also recently undertaken to identify the pathways modified by IVS and to understand how it may affect the response of mammospheres to chemotherapy. The results of these studies will give us more data to conclude regarding the interest of therapeutic fasting in vivo.

Keywords : Breast Cancer, chemotherapy, mammospheres, 2D culture

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Notes:

EFFECTIVE BRCA1/2 KNOCKDOWN IN PANCREATIC CANCER CELL LINES USING A CRISPR/CAS9-MEDIATED KNOCK-IN METHOD

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Introduction: Pancreatic cancer is one of the most difficult cancers to cure, due to a locally advanced or metastatic stage at diagnosis. The only curative treatment is surgery followed by adjuvant chemotherapy, but only concerns immediately operable tumors, i.e. 20% of patients. Chemotherapy regimens such as gemcitabine or mFOLFIRINOX protocol are the standard of care for metastatic pancreatic cancer (1). PARP inhibitors, are also used to treat metastatic pancreatic cancer in patients bearing a germline BRCA1/2 mutation (2). However, this therapeutic arsenal is not sufficient since the 5-year survival rate remains below 10% and the mortality rate close to the incidence (3). Germline BRCA mutations are found in only 4-7% of patients with metastatic pancreatic adenocarcinoma (4). Therefore, the aim of our study was to induce a BRCA1/2 mutation in human pancreatic cancer cell lines in order to increase the cell sensitivity to PARP inhibitors.
Methods: The CRISPR/Cas9-mediated knock-in technique was selected to induce deleterious BRCA1 or BRCA2 mutations in two pancreatic cancer cell lines (T3M4 and Capan-2) and a breast cancer cell line (MCF7) as control. A CRISPR/Cas9 ribonucleoprotein (RNP) was assembled for each mutation and transfected in cell lines using a lipid-based transfection reagent. The droplet digital PCR (ddPCR) was used to confirme on-target mutations and effectiveness of our CRISPR/Cas9-mediated systems. Off-target effects were predicted using CRISPR LIFEPIPE® and CrispRGold tools and off-target sites were identified by next generation sequencing (NGS).
Results: The c.763G>T (p.Glu255Ter) and c.2133C>A (p.Cys711Ter) mutations were selected to obtain truncated and non-functional BRCA1 and BRCA2 proteins respectively. Allelic frequencies obtained for BRCA1 knock-down were 91%, 55%, and 25% for MCF7, T3M4 and Capan-2 cell-lines respectively and for BRCA2 knock-down were 97%, 78%, and 20% for MCF7, T3M4 and Capan-2 cell lines respectively. No off-target effects were predicted by the different tools and no off-target sites were identified in the limit of the sequenced gene-panel.
Conclusion: We designed a CRISPR/Cas9-mediated knock-in technique to induce in vitro deleterious BRCA1/2 genes mutations in two pancreatic cancer cell-lines. Our CRISPR/Cas9 systems were able to induce BRCA1 or BRCA2 mutation in all of cell lines transfected without off-target effects. Therefore, the sensitivity to PARP-inhibitors of cells modified by the CRISPR/Cas9 system can be restored. This strategy might offer an interesting therapeutic perspective for the management pancreatic cancer. Further investigations are needed to resolve CRISPR addressing issues in in vivo models and investigate both yield and toxicity.

Keywords : Pancreatic ductal adenocarcinoma, BRCA, CRISPR/Cas9-mediated knock-in
References : Lambert A, Schwarz L, Borbath I, Henry A, Van Laethem J-L, Malka D, et al. An update on treatment options for pancreatic adenocarcinoma. Ther Adv Med Oncol. 2019;11:1758835919875568. , Golan T, Hammel P, Reni M, Van Cutsem E, Macarulla T, Hall MJ, et al. Maintenance Olaparib for Germline BRCA-Mutated Metastatic Pancreatic Cancer. N Engl J Med. 2019 25;381(4):317–27. , Rawla P, Sunkara T, Gaduputi V. Epidemiology of Pancreatic Cancer: Global Trends, Etiology and Risk Factors. World J Oncol. 2019 Feb;10(1):10–27. , Wong W, Raufi AG, Safyan RA, Bates SE, Manji GA. BRCA Mutations in Pancreas Cancer: Spectrum, Current Management, Challenges and Future Prospects. Cancer Manag Res. 2020;12:2731–42.

Notes:

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