Al collegio docenti del Dottorato in Medicina Molecolare

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Ciclo XXXVII Tutor Prof.ssa Federica Gemignani

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Malignant pleural mesothelioma (MPM) is a rare cancer with low patients' life expectancy (12 months). Targeted therapies and the use of biomarkers were proposed as promising tools to face MPM burden. Mesothelin (MSLN) is the most studied biomarker in MPM, because of its differential expression between healthy and MPM cells. Non-antibody scaffolds are an innovative strategy to improve targeted therapies and diagnosis. In this project, the binding between the tenth domain of human fibronectin type III (Fn3) and MSLN is evaluated to develop a theranostic approach against MPM.

Fn3 5.3.2 was previously engineered to bind MSLN in ovarian cancer cell lines (Kd=2.5 nM) and was provided by Prof. Sarah Moore's laboratory (Smith College). Fn3 was produced by transforming *E. coli* BL21 cells, purified through size exclusion chromatography and concentrated by dialysis. Fn3-MSLN binding was evaluated in mesothelial (negative control, Met-5A) and MPM (REN, MSTO, Mero-14) cell lines, through flow cytometry and immunofluorescence. MSLN expression in these cell lines was verified through these two techniques. Transfection of MSTO with MSLN_OHu21046C pcDNA3.1 plasmid was carried out to produce a cell line that stably overexpressed MSLN.

REN showed the highest MSLN expression level among MPM cells; therefore, we used this cell line to evaluate Fn3-MSLN binding. Fn3 5.3.2 was observed to bind MSLN with lower affinity than expected. The non-specific binding of the primary antibody, which labels Fn3, affected the analyses of the Fn3-MSLN binding signals in both immunofluorescence and flow cytometry. This non-specific signal was cell line-independent since multiple cell lines were analysed. Additionally, decreasing the antibody concentrations did not overcome this issue. In future experiments, Fn3 will be directly labelled with AlexaFluor-488, to by-pass the antibody use. MSTO clones overexpressing MSLN were selected and will be used to evaluate the binding in more detail.

Abstracts, conferences and courses

- Open Seminar IncuCyte - "Cell growth & cell health assays applications", 14/04/2022, Sartorius (seminar)
- "Genomics approaches for characterizing germline mutations in humans and animal model", 03/05/2022, Società Italiana di Mutagenesi Ambientale e Genomica (seminar)
- "Writing Scientific Paper- Lecture I. Importance of scientific writing, Title, Abstract, Introduction, Materials and methods", 17/05/2022, *Research for professional skills* course, Università di Pisa (lecture)
- Scuola di Genetica in Cortona, "Sistemi modello come strumento di analisi genetica", Associazione Genetica Italiana on 30-31 May, 2022. (Conference)
- Manuel Gentiluomo*, Margherita Piccardi*, Stefania Bertoncini, Michael Dannemann, Federico Canzian, Sergio Tofanelli, Daniele Campa, on behalf of the PANDoRA consortium. Exploring the Neandertal legacy of pancreatic ductal adenocarcinoma risk in Eurasians. Poster presentation at the 54th European Pancreatic Club Meeting 2022, 22-25 June 2022.
- Dialoghi sulla Biologia, 24/06/2022, University of Pisa (seminar)
- Ricerche bibliografiche e open access & science, Università di Siena (soft skill)
- Creating value from large archive and big data, 13/06/2022, Università di Siena (soft skill)
- I rapporti tra scienza e società: tra persistenze e cambiamenti, 15/06/2022, Università di Siena (soft skill)

- Fare ricerca per l'inclusione sociale, 27/06/2022, Università di Siena (soft skill)

Publications

- Di Franco G, Usai A, Piccardi M, Cateni P, Palmeri M, Pollina LE, Gaeta R, Marmorino F, Cremolini C, Dente L, Massolo A, Raffa V, Morelli L. Zebrafish Patient-Derived Xenograft Model to Predict Treatment Outcomes of Colorectal Cancer Patients. Biomedicines. 2022; 10(7):1474. https://doi.org/10.3390/biomedicines10071474
- Piccardi M*, Gentiluomo M*, *et al.,* 2022. Exploring the Neandertal legacy of pancreatic ductal adenocarcinoma risk in Eurasians. Submitted to *Biological Research.*

Visiting scholar experience

- From 12/09/2022 to 11/09/2023: I am pursuing research for the PhD project under the supervision of Prof. Sarah Moore, at Ford Hall, Smith College, 100 Green St, 01063, Northampton, Massachusetts, USA