Characterisation of the landscape of CD44 isoform expression and its role in modulating cell function in triple negative breast cancer (TNBC)

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Abstract: This project is about a cell surface receptor, CD44, and the mechanistic understanding of its (tumorigenic) signalling in the framework of one of current unmet needs of tumour therapy, i.e. triple negative breast cancer (TNBC). Both CD44's standard form and some of its variants are linked to tumour-initiating capacity, metastatic potential and poor patient prognosis. However, very little mechanistic insight is available in relation to their signalling, and specifically to the role of CD44 transcriptional variations on one hand, and malignancy (including the induction of Epithelial-Mesenchymal Transition, EMT) and drug responses on the other. The project includes computational and experimental activities; it spans from the analysis of public TNBC patient data to identify mechanistic hypotheses, through experimental validation in libraries of cell lines, to attempts of therapeutic intervention using combination therapies.

Rationale & Hypothesis: *Background.* CD44 is an ubiquitous surface receptor with yet an intriguing relation to tumour malignancy; its standard form is often considered a stemness marker for cancer-initiating cells and its level of expression linked to motility and metastatic potential (and the reverse applies, see Nature Medicine, 17 (2011) 211), while some isoforms strongly link to drug resistance and poor prognosis, above all in triple-negative breast cancer (see e.g. Molecular Medicine, 18 (2012) 1109; PLOS One, 7 (2012) e44078). Of note, CD44 forms are in principle 'druggable' using its main ligand - hyaluronic acid (HA) - as a carrier for a targeted therapeutic intervention. *Main questions.* What is the most 'tumorigenic' part of CD44-based signalling, e.g. which feature makes an isoform a factor of negative prognosis and/or poor drug response? Is it possible to exert a certain degree of control on TNBC phenotype using CD44 transcriptional regulation? Should we want to revert to more drug-responsive phenotypes, is CD44 signalling actionable?

Research Plan: *A) Forming the hypotheses*. Isoform-specific expression data will be extracted for triple negative tumoural and healthy breast samples from the PCAWG and GTEx projects, identifying the landscape of CD44 isoform expression across them, and grouping them according to the combinations of the expressed variable domains and transcription factor activation profiles and upstream signalling regulation (in Petsalaki group). We aim to uncover relations between peculiarities in cell signalling and gene regulatory networks and presence and expression levels of variant domains. *B) Validating the hypotheses*. The above relations will be associated with indicators of malignancy, stemness, drug resistance and metastatic potential, as extracted for these samples from the TCGA and PCAWG data repositories, thus identifying and prioritising potential subnetworks, targets or pathways. Then, a panel of human breast cancer cell lines will be used to confirm the role of these pathways, specifically probing the sensitivity of the latter to selected chemotherapeutic treatments, hypoxic conditions, and interactions of CD44 isoforms with HA (alone or in combination). *C) Refining the model*. This phase of the project will focus on establishing solid, quantitative links between expression of CD44 variants and on one hand malignancy and on the other responsiveness to treatment, with a final, in vivo verification using an orthotopic rodent model.

Integration of Expertise of Partners: *Petsalaki* group - bioinformatic analysis, with extensive experience and in-house developed tools in cell signalling, omics data analysis and integration and network analysis. *Tirelli* group - targeted drug delivery and cell-materials interactions, with in-depth expertise on CD44/HA interactions and HA-based carriers, as well as extensive access to facilities for cell culture and animal experimentation.