ROLE OF FATTY ACID AMIDE HYDROLASE (FAAH) IN BREAST DEVELOPMENT AND CANCER

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IntRODUCTION There are three major stages of breast development – embryonic, pubertal, and reproductive. During this complex developmental cycling, the epithelial compartment undergoes several rounds of proliferation, remodelling and cell death to eventually form an organised and functional mammary tree. The very nature of the pathways controlling these processes makes them susceptible to promote tumorigenic processes and ultimately lead to breast carcinoma.

Fatty acid amide hydrolase (FAAH) is an integral membrane enzyme that hydrolyzes the endocannabinoid anandamide and related amidated signalling lipids. Here, we aimed at analysing the expression FAAH in breast physio-pathology.

MATERIAL AND METHODS The expression of FAAH was analysed by immunohistochemistry in a tissue microarray (TMA) containing ~600 human breast cancer samples and in public microarrays data bases. Cell lines derived from different breast cancer subtypes have been analysed for FAAH expression and the effect of a Knock-out (CRISPR) and overexpression of FAAH examined. FACS and immunofluorescence were also performed to further assess the identity of cell populations. FAAH effects on breast CSC activity was examined using mammosphere formation and aldehyde dehydrogenase (ALDH) activity assays.

RESULTS AND DISCUSSIONS We have found that FAAH shows remarkable variations in expression within the human breast epithelial hierarchy. Particularly, FAAH expression is restricted to a certain subset of mature cells in normal breast tissue (i.e. luminal cells), with negligible levels being detected in the progenitor/stem cell subset. In line with this idea, pharmacological inhibition of FAAH with URB597 in the mammary stem cell line HC11 delayed differentiation to mature epithelial cells. In addition, we observed significant differences in duct formation between FAAH KO and WT mice during pubertal development.

Our results also correlate low FAAH expression with more undifferentiated phenotypes, high histologic grade, absence of oestrogen receptor and triple-negative phenotype in breast cancer. Consistent with this, we found low FAAH mRNA levels associated with metastasis and poor prognosis gene signatures. Finally, pharmacoinhibition and knocking-out of FAAH in breast cancer lines, enriched the cultures in cancer stem cells.

CONCLUSION Together, our data suggest that FAAH plays a role in breast cell differentiation, both in normal development and in oncologic contexts.

HETEROGENEITY OF THE ALPHAS INTEGRIN SUBUNIT EXPRESSION IN GLIOBLASTOMA

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Introduction Glioblastoma (GBM) is the most aggressive primary brain tumour. Treatment failure and recurrence are explained by intratumoral heterogeneity. Our previous results showed that the integrin α5β1, the fibronectin receptor, is implicated in GBM aggressiveness and represents a pertinent therapeutic target.

Recently, we observed that its expression was heterogeneous between patient tumours but also between different areas in a given tumour. We hypothesised that this intratumoral heterogeneity may be linked to different glioma initiating cells (GIC).

MATERIAL AND METHODS GICs were grown as neurospheres in stem cell medium and their differentiation was induced by serum. We characterised α5β1 expression in 9 GICs cell lines before and after differentiation. Two cell lines were selected and were genetically modified by depletion (CrisprCas9) or transfection of the α5 integrin gene. Different clones were selected expressing or not the integrin. Aggressiveness of polyclonal lines and individual clones was analysed in vitro before and after differentiation (proliferation, migration, evasion from spheroids) and in vivo (orthotopically xenografted cells).

RESULTS AND DISCUSSIONS Our results show that α5 integrin is not expressed in stem cell culture conditions. However, α5 expression is induced after differentiation in about half of the cell lines supporting the notion of inter-tumoral heterogeneity of GICs. Interestingly, single cell-derived clone evaluation showed that intra-tumoral GICs heterogeneity also exists. We noticed that when GICs are programmed or forced to express α5 integrin, differentiated cells became more aggressive. Notably, differentiated cells, expressing the integrin, acquired a fibronectin-dependent motility and a proliferative phenotype. The in vivo assays demonstrated that GICs, programmed to express the integrin, were prone to form larger tumours.

CONCLUSION Our data support the hypothesis that some GICs are programmed to express the α5 integrin subunit to form a more aggressive tumour. Further studies will be needed to explore the implication of such heterogeneity in resistance to anti-integrin therapies but also to conventional chemo/radiotherapies.

ELUCIDATING THE POTENTIAL ROLE OF CD109 AS A BIOMARKER FOR CANCER STEM-LIKE CELLS IN CERVICAL CANCER

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IntRODUCTION Cervical cancer is a common genital tract cancer. Radiotherapy is the mainstay of management for advanced cervical cancer. Response to radiation varies widely which may be explained by the existence of cancer stem-like cells (CSCs). Since CSCs is implicated in cervical cancer and demonstrated a high degree of resistance to radiation, the identification of novel CSC markers could be critical to specifically target the cervical CSC. In our pilot study, we established attached and spheroid cells from primary cervical tumour tissue. Multiple ‘stemness’ genes were detected in spheroid cells which indicated primary cervical cancer tissue harboured CSCs population. cDNA microarray analysis was performed to compare cDNA expression profile and CD109 was significantly up-regulated in spheroid cells. Therefore, we hypothesised that CD109 may serve as a potential cervical CSC marker.

MATERIAL AND METHODS Flow cytometry was performed to analyse the CD109 expression and isolate the CD109 positive...
and negative sub-populations in cervical cancer cells. Following the sorting, cell proliferation and migration assay were performed. In order to investigate the effect on the blockade of CD109, SiHa, Caski and C4-1 cells were transfected with CD109 siRNAs. XTT assay, migration and invasion assay and spheroid formation assay were performed. Immunohistochemistry (IHC) was performed for the detection of CD109 expression in cervical cancer tissue microarray (TMA).

Results and discussions The post-sorted CD109 (+) cells grew remarkably faster and have stronger migration capability than CD109(−) cells in Caski and C4-1. The CD109 knockdown cells with siRNA exhibited a slower cell growth, decreasing migration and self-renewal ability, as compared with the control group in SiHa, Caski and C4-1 cells. IHC of TMA indicated that CD109 was highly expressed in cancer cases than that in normal/benign cases.

Conclusion CD109 increased cell proliferation rate and migration ability in post-sorted cervical cancer cell lines Caski and C4-1. On the contrary, CD109 knockdown reduced cell growth, migration and self-renewal capability in cervical cancer SiHa, Caski and C4-1 cells. Cervical carcinoma showed high expression of CD109 protein by IHC. Further in vitro and in vivo functional assays are essential to characterise the CD109-positive sub-population in cervical cancer which may provide more information of cervical CSCs-related properties and resistance of radiation therapy, with the underlying molecular mechanism involved.

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ONCOGENIC ACTIVITY OF SOX1 IN GASTRIC CANCER

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Introduction Gastric cancer (GC) remains one of the leading causes of global cancer mortality due to therapy resistance. The infection with Helicobacter pylori is the major risk factor, particularly in patients carrying H. pylori highly virulent strains. GC contains a sub-population of gastric cancer stem cells (gCSC) with capacity of self-renewal that are critical drivers of tumour initiation, recurrence and therapy resistance. SOX transcription factors play a key role in the regulation and maintenance of stem cells during the embryonic development and are also involved in cancer. SOX1 is a well-established tumour suppressor in several types of cancer and has an oncogenic role in glioblastoma, but its impact in GC remains largely unknown. Therefore, we aimed to elucidate its function in GC and its putative role in the action of H. pylori.

Material and methods For this, we analysed GC patient samples, we co-cultured human GC cell lines with different H. pylori strains derived from patients of Donostia Hospital and we also performed functional studies of gain and loss of SOX1 function. Also, we performed transcriptomic analysis in SOX1-silenced cells and we carried out computational analysis using the ACRG datasets.

Results and discussions Our results revealed that SOX1 is highly expressed in human GC samples. Moreover, among a subset of SOX genes, SOX1 was the most significantly up-regulated in gCSC derived from GC cell lines and also in cisplatin resistant cells, as well as in response to H. pylori exposure, in a virulence-dependent manner. In GC cells, SOX1-silencing impaired self-renewal capacity in vitro and reduced tumorigenicity and tumour growth in vivo. The up-regulation of SOX1 showed the opposite phenotype, indicating that SOX1 exerts an oncogenic role in GC. Notably, we found that in GC cells SOX1 was required for H. pylori-induced proliferation, acquisition of stem cell-like properties and induction of β-catenin. Furthermore, the transcriptomic analysis revealed a significant alteration of the E2F signalling pathway in SOX1 knockdown cells. Consistently, E2F1 silencing phenocopied SOX1 knockdown. Moreover, the inhibition of SOX1 downregulated E2F1, suggesting that E2F1 could be a downstream effector of SOX1 in GC.

Conclusion We identify for the first time an oncogenic role of SOX1 in GC. Our findings establish that SOX1-E2F-b-catenin is a critical axis for gCSC maintenance and for H. pylori action, postulating that its inhibition could constitute a promising strategy to combat therapy resistance in GC.

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MYC FAVOURS THE ONSET OF TUMOUR INITIATING CELLS BY INDUCING EPIGENETIC REPROGRAMMING OF MAMMARY EPITHELIAL CELLS TOWARDS A STEM CELL-LIKE STATE

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Introduction Breast cancer consists of highly heterogeneous tumours whose cell of origin resulted difficult to be defined. Recent findings highlighted the possibility that tumor-initiating cells (TICs) may arise from dedifferentiation of lineage-committed cells, by reactivation of multipotency in response to oncogenic insults. MYC is the most frequently amplified oncogene in breast cancer and the activation of MYC pathway has been associated with the basal-like subtype, which is characterised by poor survival and lack of a specific therapeutic strategy. Although MYC has been considered a driver oncogene in breast cancer, its mechanism of action in tumour initiation has been poorly addressed.

Material and methods To evaluate the role of MYC in perturbing cell identity of somatic cells, we transduced hTERT-immortalised human mammary epithelial cells (IMEC) with a retroviral vector expressing low levels of the exogenous c-Myc. The effect of MYC overexpression was evaluated by performing morphological analysis and gene expression profiling. To verify whether MYC overexpression could enrich for cells with functional stem cell-like properties, we performed mammospheres assay. ChIP-seq analyses were performed to profile chromatin modifications and MYC binding in IMEC WT, -MYC and mammospheres. To determine whether MYC-reprogrammed IMEC were enriched for TICs, we performed in vivo injection in NOD/SCID mice and assessed long-term tumorigenic potential by performing serial transplantation assay. To assess the clinical relevance of our findings, we investigated the expression of MYC-dependent oncogenic signature in a database of breast cancer patients.

Results and discussions Overexpression of MYC induces transcriptional repression of lineage-specifying transcription factors, causing decommitting of luminal-specific enhancers. Of note, MYC-driven dedifferentiation supports the onset of a