**PO-294** 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 examinated 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.

**PO-295** HETEROGENEITY OF THE ALPHAS INTEGRIN SUBUNIT EXPRESSION IN GliOMAstoma

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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 (orthotypically 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.

**PO-296** 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 CSCs marker.

**Material and methods** Flow cytometry was performed to analyse the CD109 expression and isolate the CD109 positive cells.