Breast Cancer RESEARCH

MEETING REPORT

'The charmingest place': non-coding RNA, lineage tracing, tumor heterogeneity, metastasis and metabolism - new methods in mammary gland development and cancer: the fifth ENBDC Workshop

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Abstract

The European Network for Breast Development and Cancer (ENBDC) Workshop on 'Methods in Mammary Gland Development and Cancer' has grown into the essential, international technical discussion forum for scientists with interests in the normal and neoplastic breast. The fifth ENBDC meeting was held in Weggis, Switzerland in April, 2013, and focussed on emerging, state-of-the-art techniques for the study of non-coding RNA, lineage tracing, tumor heterogeneity, metastasis and metabolism.

Introduction

Mark Twain once described Weggis, our workshop location, as '…the charmingest place we have ever lived in for repose and restfulness'. Participants in the fifth European Network for Breast Development and Cancer (ENBDC) Workshop on 'Methods in Mammary Gland Development and Cancer' were equally charmed but likewise invigorated and excited by the high quality of the keynote and invited speakers for the three day meeting.

Non-coding RNA Keynote Lecture and metastasis session (Chair: Rob Clarke)

We were very privileged to have Professor Jeff Rosen from Baylor College of Medicine, Houston, to open the ENBDC workshop with a talk on the exciting and little researched area of long non-coding RNAs (lncRNAs). lncRNAs are involved in many aspects of gene regulation, such as XIST, which targets the polycomb complex PRC2 to inactive X chromosomes. They can act as enhancers, epigenetic modifiers or repressors of gene transcriptional activity. One example is called Hotair, which binds and targets PRC2 to the HoxD locus. Another example is called Malat 1, which is highly expressed in luminal breast tumors. Ten thousand known lncRNAs show differential expression across molecular subtypes of breast cancer. Jeff told the workshop about his discovery of a pregnancy-induced lncRNA (PINC), which is sustained after involution of the mammary gland post-lactation [1]. PINC is mammalian-specific and contains no conserved open reading frames, although small peptides may be encoded by it. There are at least eight major splice forms of mouse PINC and it is increased during pregnancy, specifically in luminal cells. PINC knock down in mammary cells increases lactogenic differentiation. Co-precipitation experiments show that mouse PINC transcripts interact with PRC2 proteins and affect the gene expression of approximately 400 genes, of which approximately 80% are repressed [2].

A young researcher, Dr Albert Santamaria-Martínez, from the laboratory of Prof. Joerg Huelsken in Lausanne, presented in the metastasis session. Albert is working on the MMTV-polyoma middle (PyMT) model of breast cancer, which is known to spontaneously metastasize to the lungs in approximately 90% of mice. In order to study the process of lung colonization, they crossed the PyMT with an actin-green fluorescent protein (GFP) mouse so that cells could be tracked *in vivo*. CD24 + CD90+ cancer stem cells (CSCs) were FACSorted and injected via the tail vein to evaluate metastatic capacity.

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They found that only CSCs are able to form metastatic nodules. Next, the group aimed at identifying microenvironmental factors that are crucial for metastatic colonization of CSCs. To address this question, the group performed microarray analyses by extracting RNA from microdissected normal stem cell niches and comparing it to their differentiated epithelium and adjacent stroma counterparts. They did the same comparing metastatic cancer cells and the reactive stroma. They discovered periostin (POSTN) around elongating mammary gland terminal end buds and in fibroblasts surrounding PyMT tumors. By generating a POSTN knockout mouse and crossing it with the PyMT model, they showed that POSTN knockout mice have reduced lung metastases. Interestingly, there were also lower numbers of CSCs in the few lung metastases that formed. Proteomics demonstrated that POSTN binds Wnt ligands and a 20xLEF-GFP Wnt reporter transduced into PyMT tumor cells showed that CSCs have the most Wnt signaling activity. In turn, transforming growth factor (TGF) β3 secreted from the colonizing tumor cells is a strong inducer of POSTN production by the stroma. In summary, CSCs reach the secondary site and secrete factors such as TGFβ3, inducing POSTN expression; POSTN in turn binds Wnt ligands that are presented to the CSCs so they can self-renew and survive in the new, harsh environment [3].

Lineage tracing in the mammary gland session (Chair: John Stingl)

Renée van Amerongen discussed her experiments using an Axin2CreERT2 knock-in mouse and showed how this lineage-tracing model allowed her to mark different populations of Wnt/beta-catenin-responsive cells at distinct stages of mammary gland development [4]. These analyses not only supported the co-existence of unipotent and multipotent stem cells in the mammary epithelium, as evidenced by their ability to survive complete turnover of the mammary epithelium and contribute to the building of alveolar structures during multiple rounds of pregnancy, but also revealed an important difference between the normal developmental potential of a given cell population *in situ* and the regenerative potential of the same cell population tested by transplantation.

Although lineage tracing thus offers unique possibilities in terms of investigating how the mammary epithelium is built and maintained, Renée also highlighted some of the practical considerations associated with this approach. She pointed out how tamoxifen-mediated recombination in the mammary gland is quite inefficient compared to other tissues, in particular when using a multi-color reporter such as the Rosa26Confetti allele developed by Hans Clevers [5]. While this increases the likelihood of studying clonal events, it makes it more difficult to perform detailed quantifiable analyses.

Alexandra Van Keymeulen (Universite Libre de Bruxelles) gave a presentation describing her work describing luminal and basal stem cell populations in the mouse mammary gland [6]. Transplantation of mammary epithelial cells into cleared mammary fat pads of primary and secondary mice has historically been used as an assay to detect cells that have the ability to recapitulate all the elements of the mammary epithelium and self-renew [7,8]. The cells that had the ability to generate these outgrowths have been termed mammary repopulating units (MRUs) and are described as having a basal phenotype [9-11]. This MRU assay, when conducted at a clonal level, was perceived as the 'gold standard' assay for the detection of mouse mammary stem cells. However, Alexandra Van Keymeulen and colleagues used an inducible lineage-tracing strategy in which cell lineage-specific promotors (for example, keratin (K)5, K14, K8 and K18) were used to direct expression of Cre recombinase to specific subsets of mammary epithelial cells such that these cells and their progeny are irreversibly marked with a reporter gene. By the use of such a strategy, Alexandra Van Keymeulen was able to demonstrate that the luminal and basal cell compartments are maintained, in both the resting state and during pregnancy, by their own stem cell pools. This is in marked contrast to previous results that demonstrated that MRUs have multilineage potential, whereas luminal epithelial cells were reported to lack stem cell potential [9-11]. During the discussion session the topic turned to a recent report from the Werb laboratory that Lgr5 identifies MRUs in the mouse mammary gland [12]. However, neither Alexandra Van Keymeulen (unpublished) nor Jos Jonkers [13] have been able to reproduce these findings since they both observed that the Lgr5-subsets of mammary epithelial cells contain MRU activity. Unfortunately, no satisfactory conclusion was reached during the discussion session as to why different laboratories are obtaining such conflicting results.

Breast tumor heterogeneity session (Chair: Momo Bentires-Alj)

Peter Van Loo from the Cancer Genome Project, Wellcome Trust Sanger Institute, Cambridge, UK, and the Department of Human Genetics, VIB and University of Leuven, Belgium, spoke on 'Genomic ventures into inter- and intra-tumor heterogeneity of breast cancer'. He highlighted what studies on the genomics of breast cancer have taught us about inter-tumor heterogeneity, the differences and similarities between breast cancers, and intra-tumor heterogeneity, subclonal architecture and tumor evolution.

He first described studies from the Børresen-Dale group showing that breast tumor classifications built on levels of genomic distortion have prognostic value [14]. Van Loo then described work from the Cancer Genome Project that identified the landscape of driver mutations in 100 breast cancers using exome sequencing [15]. Notably, they found 73 different combinations of mutated cancer genes, highlighting the considerable genetic diversity of breast cancer and suggesting that - when the combination of driver mutations is used to describe breast cancer - almost every breast cancer is unique. They also investigated the subclonal architecture and the evolution of breast cancer by performing whole genome sequencing on 21 breast cancers and developing several bioinformatics algorithms to unravel the life history of these tumors [16]. They showed that subclonal diversification is prominent, and most mutations are found in just a fraction of tumor cells. Every tumor has a dominant subclonal lineage representing more than 50% of tumor cells and expansion of the dominant subclone to an appreciable size triggers diagnosis. Van Loo concluded that, in the near future, the genomic landscape and the evolutionary process of a patient's breast cancer will be able to be characterized and should help to design a personalized therapy.

Cancer metabolism session (Chair: Maria dM Vivanco)

Tumors reside in complex microenvironments. To understand fully the behavior of carcinoma cells it is important to consider their interactions with the cells around them and the influence that the microenvironment exerts on the tumor cells. Michael Lisanti spoke about how stromal 'cancer associated fibroblasts' (CAFs) can promote tumor growth. He discussed data supporting the so-called 'reverse Warburg effect' or two-compartment tumor metabolism, whereby oxidative stress in CAFs elicits autophagy leading to the onset of aerobic glycolysis in the tumor stroma. This results in the production of high-energy nutrients by the CAFs leading to oxidative metabolism in cancer cells. In addition, he showed that CAFs secrete greater levels of growth factors, extracellular matrix components and matrix metalloproteinases than normal fibroblasts, and that this promotes the ability of epithelial cells to invade the surrounding stroma. Dr Lisanti spoke about MCT4, a functional marker of hypoxia and oxidative stress, as a biomarker that predicts poor clinical outcome in triple-negative breast cancers only when highly expressed in the stroma, particularly if combined with the loss of stromal caveolin-1 [17]. The future development of MCT4 inhibitors to treat breast cancer tumors of this type, which have poor prognosis, could represent an attractive therapeutic approach. To illustrate the potential therapeutic benefit of targeting CAFs, Dr Lisanti discussed tumor necrosis factor-α function as a tumor suppressor and its stromal cell-mediated delivery to prevent breast cancer tumor formation in mouse xenografts [18]. In conclusion, this talk emphasized the potential of the tumor stroma as a resource for new biomarkers and also as a target for new forms of therapy.

In the second presentation, Dimitrios Anastasiou discussed current approaches his laboratory is using to improve our understanding of how nutrient metabolism differs between tumor and normal cells and how this contributes to cancer progression. Oncogenic mutations may be implicated in the 'metabolic reprograming' that allows cancer cells to use nutrients in a different manner to support their own cell proliferation. Metabolic reprograming includes the use of alternative enzyme isoforms with distinct regulatory properties. One of these examples is the glycolytic enzyme pyruvate kinase, whose M1 isoform (PKM1) is commonly found in normal tissues, while the alternatively spliced form PKM2 is found in embryonic tissues and tumor cells. Dr Anastasiou spoke about the use of small-molecule PKM2 activators to promote a constitutively active enzyme that leads to the growth inhibition of xenograft tumors and that could be used to interfere with anabolic metabolism [19]. In addition, he showed that inhibition of PKM2 might promote cancer cell survival and tumor growth under oxidative stress [20]. Thus, despite the many challenges facing metabolism research, it may be possible, as suggested by the use of small molecule PKM2 activators, to target cancer metabolism in the future for therapeutic purposes.

Conclusion

In addition to the invited talks described above, we heard eight selected short talks of excellent quality and covering a wide range of topics in mammary gland biology and cancer. We also had two poster sessions going on well into the evening, which was testament to the interest that they aroused. Overall, the ENBDC annual meeting once again proved itself to be an outstanding international forum for discussion on mammary gland and breast cancer research.

In order to return to Weggis, this most 'charmingest place', organization of the next ENBDC meeting, on 8–10 May 2014, has already begun.

Abbreviations

CAF: Cancer associated fibroblast; CSC: Cancer stem cell; ENBDC: European Network for Breast Development and Cancer; GFP: Green fluorescent protein; lncRNA: Long non-coding RNA; MRU: Mammary repopulating unit; PINC: Pregnancy-induced lncRNA; PyMT: MMTV-polyoma middle; TGF: Transforming growth factor.

Competing interests

The authors declare that they have no competing interests.

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