Mammary Gland in Development & Cancer

Guest Editors

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Preface

Developmental and Cancer Research on the Mammary Gland Nowadays

In this Special Issue, a number of common biological mechanisms are presented that govern both mammary gland development and mammary cancer. About half of the contributors present data on normal growth and differentiation of the gland, while for the others, cancer is the dominating theme. Yet, nearly all of them cross-refer to both research fields, and one of the innovating concepts in this respect is the existence of cellular ecosystems operating in development and cancer. The introductory paper by Marc Mareel and Susana Constantino already underpins the importance of the ecosystem concept by pointing to the existence of cell-cell interactions at multiple levels: between epithelial cells with different phenotypes (stem cells, non-stem cells, ductal cells, myoepithelial cells, etc.), between epithelial cells and stromal elements (endothelial cells, cancer-associated fibroblasts, macrophages, etc.) and, last but not least, between different ecosystems within the organism (primary tumor, metastases, bone marrow, etc.). Strikingly, the research contributions from other authors in this Issue add numerous ecosystem examples in development and cancer, confirming their general occurrence in mammary biology. Historical background information on the ecosystem concept is further provided in the interview with Marc Mareel, translating its relevance also for cancer therapy.

Another central issue is the stem cell. Thus, the role of normal adult stem cells in growth and differentiation of the mammary gland is considered from different points of view. Agla Fridriksdottir *et al.* pay attention to markers for identification and localization of these cells in the mammary gland and warn for possible differences between murine and human data. Here again, the interaction between stem cells and the stromal microenvironment cannot be overestimated. A unique approach is presented by Maria Ferletta *et al*. who use a cell line established from a benign canine mammary tumor for flowcytometric sorting of the "side population", which appears to express stem cell markers (CD 44+, CD 49F+, CD 24-). Remarkably, the sorted cells efficiently form spheroids that express sox2 and oct4. Two interesting questions are discussed by Vassiliki Pelekanou and Guy Leclercq: do normal stem cells express estrogen receptors? and: does cancer directly derive from normal stem cells that become mutated, or from cancer stem cells generated by a mutated epithelium? Growth and regeneration of mammary gland epithelium is further sustained by delayed apoptosis and the intra-epithelial crosstalk with myoepithelial cells. To understand the action mechanism of the anti-apoptotic receptor in mammary gland epithelium, Jean-Philippe Wilmet *et al.* use a model with TRAIL-induced apoptosis, and observe that overexpression of the anti-apoptotic neurotrophin receptor $p75^{NTR}$ can block this apoptosis. Using proteomics, they discovered that overexpression of the receptor downregulates HSP 27, a pro-apoptotic protein in mammary epithelium. The functions of myoepithelial cells in the normal mammary gland are described by Mejdi Moumen *et al.;* they are implicated in epithelial regeneration and differentiate to contractile cells, while continuously receiving signals from the extracellular matrix and from neighbouring cells. Markers indicate that basal-type mammary carcinomas have a common origin with the myoepithelial cells.

Three contributions focus on key molecules in normal mammary development (but in mammary cancer progression as well). First, Sara McNally *et al.*, demonstrate the role of JNK activity in epithelial acinus formation. The transcription factor controls this process at multiple phases, as demonstrated in murine and human three-dimensional cell models. The paper dissects these phases in detail (proliferation, acinus formation, lumen clearance etc.), and the model with differentiating non-cancer cells (MCF-10A) closely resembles the one with MCF-7 mammary cancer cells used by the research team of Marc Bracke. Second, the function of connexins in mammary development and cancer is highlighted

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by Jamal El Saghir *et al.* Although these proteins seem to serve a unique goal, namely gap junction formation and related intercellular communication, they can display a high functional variability thanks to the existence of multiple isoforms and phosphorylation patterns, and to connexon assembly heterogeneity. Third, Sureshbabu *et al.* focus on new insights into the contribution of insulin-like growth factor binding proteins to mammary gland development. The data bring the function of these binding proteins beyond their known insulin-like growth factor (IGF)-I sequestrating/modulating capacities to a new concept of binding the IGF-I/IGF-binding protein (IGFBP) complexes to extracellular matrix components. The IGFBPs are considered here as members of the extracellular/matricellular CCN-family, which is important in mammary gland morphogenesis and remodeling.

Not only can factors involved in the growth and development of the mammary gland contribute to our understanding of mammary cancer. The study of the physiological involution of the gland (most prominent after pregnancy) is also relevant to both developmental and cancer biologists. This process of involution of the mammary gland is a complex event, characterized by extensive death of the secretory epithelium, coupled with remodeling of the extracellular matrix and adipogenesis to regenerate the fat pad. Associated with these events is an inflammatory cascade and an acute phase response. While these remodeling mechanisms of the mammary gland are treated in detail by Christine Watson and Peter Kreuzaler, the paper by Jenean O'Brien *et al.* points to the pro-tumorigenic postpartum mammary microenvironment. Importantly, non-steroidal anti-inflammatory drugs appear to reduce the risk of developing mammary cancer during the postpartum period.

In line with other malignant tumors, mammary cancer invasion is dependent on modulation of cell-cell and cell-extracellular matrix (ECM) adhesion, and on directional cell motility. E-cadherin downregulation and N-cadherin upregulation have been reviewed previously in relation to the acquisition of mammary cancer invasiveness. In the present Issue, André Albergaria *et al.* focus on P-cadherin. This transmembrane molecule can be considered as a stem cell marker, and is expressed in undifferentiated normal epithelial cells and in poorly differentiated mammary cancers. Post-translationally, its activity can be regulated both extracellularly via the generation of soluble P-cadherin ectodomains and intracellulary via $p120^{CTN}$. During ductal branching morphogenesis, P-cadherin is expressed at the cap cells of the end buds, while in adult mammary gland tissue, it is detectable only in the myoepithelial cell layer. Another adhesion molecule implicated in mammary tumor progression is galectin-3, as shown by Joana Tavares de Oliveira *et al.* Thus, loss of galectin-3 and sialylation-related masking of its ligands, in conjunction with their overexpression in specific tumor cell subpopulations, are crucial in regulating adhesive/de-adhesive events in the invasive process of mammary cancer cells. In the contribution of Lara Derycke *et al.,* the contractility protein myosin IIA is put forward as a crucial denominator of MCF-7 mammary cancer cell invasion in organotypic three-dimensional confronting cultures. Here, blebbistatin appears to be a very specific and potent inhibitor of both myosin II activity and MCF-7 invasion.

Morphological evidence of host participation in the primary tumor, the pre-metastatic niche and metastatic sites are: desmoplasia consisting of myofibroblasts and ECM, and inflammation and immune response represented by lymphocytes and macrophages. Breast carcinoma-associated myofibroblasts are believed to have 3 major distinct origins, which are not necessary exclusive: resident fibroblasts, marrow-derived mesenchymal stem cells and resident stromal precursors, such as endothelial cell, pericytes and adipocytes. Abdelilah Aboussekhra makes a round-up of myofibroblast-derived paracrine factors, and their permissive roles in the growth and invasion of mammary cancer cells. These interactions are the basis of their prognostic value and their possible targets for new therapies. Infiltration of the tumor microenvironment by macrophages is associated with poor outcomes in breast cancer and other solid tumors. However, the specific identity of the macrophages and the role of many of the soluble factors these macrophages produce remains to be elucidated in detail. Damya Laoui *et al.* point to the existence of at least two distinct tumor-associated macrophage (TAM) subpopulations in mammary tumors based on a differential expression of markers such as CD206 or MHC II, and different *in vivo* behaviour: perivascular, migratory TAM which are less M2-like, and sessile TAM found at tumor-stroma borders and/or hypoxic regions that resemble more M2-like or "trophic" macrophages. Hence, a further refinement of the molecular and functional heterogeneity of TAM paracrine factors from tumor-associated macrophages to and from mammary cancer cells are described. By extension, the proinflammatory environment associated with obesity specifically highlights the involvement of obesity-associated hormones/growth factors in cross-signaling between macrophages, adipocytes and epithelial cells in mammary cancer aggressiveness. In accordance

with the data from Jianxiang Tan *et al.*, adipocytes drive the development of the mammary gland, and establish a molecular cross-talk between cancer-associated adipocytes and invasive cancer cells. These adipocytes secrete motility factors and can delipidate, presumably to provide material suitable for integration into lipid membranes of the cancer cells. As a consequence, dilipidated adipocytes may differentiate into tumor-associated myofibroblasts. The impact of endocrine estrogenic modulators on mammary gland cells is reviewed by Vassiliki Pelekanou and Guy Leclercq. Both endogenous estrogens and phyto/xenoestrogens influence mammary development and cancer behavior. The role of α- and β-estrogen receptors can be direct or indirect, and cross-signaling between these receptors and peptide factor membrane receptors, such as tyrosine kinase growth factor receptors, are considered as major estrogen effect interactors. The interview with G. Jordan revealed his pioneering work to introduce estrogen receptor targeting in the clinic. His skills for translational research convinced the pharmaceutical industry to test a failed morning-after pill to become tamoxifen, the first drug to be approved in the United States of America by the Food & Drug Administration (FDA) for the reduction of the incidence of breast cancer in high risk pre- and postmenopausal women.

The second introductory paper of the Issue by Sue Eccless describes the complex growth factor tyrosine kinase receptor (EGFR/Erb-B), its signal transduction and paracrine interactions between mammary epithelial cells and the surrounding stroma to direct proliferation, duct formation, branching and terminal differentiation during puberty, pregnancy and lactation. Caricatures of the normal role of EGFR/Erb-B signalling resulting in aberrant cellular responses are seen in breast cancers. Therapeutics challenging EGFR/Erb-B have shown significant clinical responses in some breast cancer subtypes. Complex molecular networks connected with signal transduction and new therapeutic designs are further explained in detail by Christian Gespach in the second interview of this Issue.

Exosomes are vesicles of endocytic origin released by many cells. These vesicles can mediate communication between cells, facilitating processes such as antigen presentation. Exosomes have huge implications for regulating biological function. Emerging data show involvement of exosomes in vastly different diseases including Alzheimer, macular degeneration, HIV infection, cancer, allergies and so on. In the breast, exosomes are produced by normal mammary epithelial cells and cancer cells, which may protect the gland against bacterial infection, drive gland development and cancer dissemination. An Hendrix and Alistair Hume suggest that exosomes can transport and disseminate tumor lipids, proteins, RNA types and DNA material systemically, so their role in metastasis formation is hypothesized. In the paper by Zafira Castano *et al.*, a review of the generation of data in mice leads them to propose that certain mammary tumors can stimulate the growth of a second, otherwise quiescent or indolent tumor in the same animal, by stimulating stromal formation. These data shed new light on the importance of bone marrow in tumor growth and the role of osteopontin and granulin in carcinogenesis. Systemic regulation of mammary cancer will reveal new therapeutic targets in oncology.

Our lab was fortunate to host Prof. Juan Aréchaga for a one-month visit during the Summer of 2010. He arrived during the ten-day-long "Ghent Festival" (*Gentse Feesten* in Dutch)! Over the next few days, several dedicated lab members explained to him their PhD projects. As a developmental biologist and pathologist, Prof. Aréchaga joined our weekly staff meetings and learned about the technical details of several functional models used in cancer research. Breast cancer is a central theme in the lab with a focus on invasion, cadherins, myosins, mesenchymal host cells and vesicle transport. During those moments, the idea arose to prepare a Special Issue of *The International Journal of Developmental Biology* devoted to the Mammary Gland in Development and Cancer. It was a great pleasure for us to act as Guest Editors of this Special Issue, and we appreciate all the gracious help from the *Int. J. Dev. Biol.* Editorial Team in getting this Issue together.

Finally, we would like to give special thanks to the authors of the articles compiled in this Special Issue for their generous contributions and for dedicating their valuable time. The original experimental papers are of high quality. The reviews of rapidly moving fields are thorough and insightful and have taken a lot of work to prepare. Our gratitude goes to all authors for their contributions to this scientific tale.

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