



2006 Research Grant Program Winning Abstract

Mammary Cancer Fate is Determined in the Pre-Cancer Stem Cell

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The early origins of breast cancer have become the subject of intense interest. In some cancers, colon cancer, for example, there is a clear stepwise progression with morphologically recognizable intermediate stages correlated to precise molecular alterations. In mammary cancer, epidemiologic and molecular evidence points to early evolutionary divergence and cancer fate commitment. We hypothesize that all of the changes committing a mammary cell to become a cancer occur before mammary intraepithelial neoplasia lesion is detectable. Our evidence shows that these changes commit the cell to become an invasive cancer with specific behavioral properties. We also hypothesize that this commitment does not depend on additional molecular “hits” and that the path of commitment is not altered by additional molecular “hits”. Therefore, there must be progenitor cells that encode the genetic (or epigenetic) information required for these behavioral properties. The encoded information should be different for progenitor cells with different malignant potentials and all progeny of a given progenitor should show parallel evolution. Newly proposed models invoke the concept of cancer “stem cells” which may be present in very small numbers until “re-initiation” results in recognizable pre-cancer (DCIS) and invasive carcinoma. Ultimately, even if they are identified, the biologic potential of these early “stem cells” cannot be assessed empirically in humans. Mouse models of early mammary neoplasia permit experimental evaluation of biologic potential of specific tissues and cells, and can be genetically reengineered.

In Cardiff laboratory have been isolated the pre-cancer stage from genetically engineered mouse mammary gland, and characterized the morphology and behavior. These are derived from the FVB Tg(PyV-mT) mouse, the best validated model of accurate molecular and phenotypic pathogenesis compared to human breast carcinoma, particularly high grade, metastasizing, and ERB2 amplified carcinomas. These isolated mammary tissues meet experimental criteria for pre-cancer, mimic key features of human DCIS, and are highly consistent and reproducible. Because they represent experimentally defined pre-cancer we have called them mammary intraepithelial neoplasia outgrowths (MIN-O). This serial transplantation of the mammary pre-cancer tissues shows highly consistent latency to invasive carcinoma over 40 generations. Genetic susceptibility and environmental factors are excluded in these genetically identical mice. Furthermore, only three of six lines develop metastatic carcinomas. Six heterogeneous lines of mouse mammary pre-cancer behave consistently with respect to properties of latency to tumor formation and metastasis. This would seem to contradict the “multi-hit” hypothesis, whereby a pre-cancer would require one of a number of additional hits in order to fully transform, and metastasize. The most likely conclusion is that there are progenitor cells in the serially transplanted pre-cancer tissue that carry all of the necessary information to 1) differentiate, 2) transform into carcinoma at a specific time, and 3) give rise to either a metastatic or non-metastatic carcinoma. This mouse model system provides a window on the early evolution of mammary cancer.



The goal of this study is to identify, isolate, and characterize these progenitor cells, pre-cancer stem cell populations, to study their behavior, and to discover the mechanisms of imprinted/encoded cancer fate.

In this project, we sought to optimize the transfer of normal, premalignant (MINO) and invasive tumor mammary tissue to suspension cultures and test the biology of these cultured tissues by retransplantation.

We want characterize the cell types in mammary pre-cancer using immunohistochemistry for stem, luminal, and myoepithelial markers and flow cytometry and cell sorting with a battery of BD antibody stem cells marker as CD24, CD29, CD49f. The resulting FACS sorted and collected cells will be isolated and independently retransplanted into gland cleared mammary fat pads of FVB mice to understand the biologic potential of specific cell subsets. Transplantation of any of the epithelial cell populations enriched by flow cytometry may result in either the consistent phenotype of the parent MIN-O tissue, or a new phenotype. The latency of this tumor development will be assessed and compared. If the transplantation of a specific cell population results in a tumor phenotype without associated MIN-O phenotype growth, and a shortened latency, this will be evidence for the role of the tissue context in regulation of transformation. Experimental comparisons to the tumors and MIN-Os in our preliminary data will be made, including DNA content by array based comparative genomic hybridization, gene expression profiles by oligonucleotide array, and the histologic and immunohistochemical phenotype.

The 3D culture of our MINO-model allows also in vitro transfection of normal and premalignant mammary tissue to elucidate which molecular pathways are necessary or sufficient for malignant transformation. Using highly efficient viral vector systems MIN-O transplants will be engineered to carry reporters, candidate gene overexpression, “knock- downs”, and gene libraries with selectable endpoints for gene discovery to understand the biologic effect in mammary pre-cancer.

Our Mouse model provides the only model currently available to identify a pre-cancer stem/progenitor cell population. Our data shows that such a population is present. Isolation, molecular characterization, and genetic manipulation of these cells promises a new understanding of mammary carcinoma origins, challenges the dogma of multi-hit progression, and confirms the early emergence of the pre-cancer stem cell as the origin of breast cancer.

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