Wednesday, 22 March 2006 11:15–12:45

PLENARY KEYNOTE

Research

1 Invited

Hunting for breast cancer genes

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First degree relatives of women affected by breast cancer have an approximately two-fold risk of developing the disease. This excess familial risk is predominantly attributable to inherited susceptibility to the disease. Mutations in *BRCA1* and *BRCA2* account for approximately 20% of the familial risk of breast cancer, therefore 80% remains to be explained. The hunt for these additional breast cancer susceptibility genes is being conducted in three ways: genetic linkage analyses; association studies; and mutational screens of candidate genes. The progress and results of these strategies will be reviewed.

2 Invited

Stem cells in breast development and cancer: implications for prevention and therapy

M. Wicha. *University of Michigan Comprehensive Cancer Center, Ann Arbor, USA*

The mammary gland epithelial components are thought to arise from stem cells which have a unique capacity for self-renewal as well as for differentiation into the lineages which comprise the lobular alveolar structure of the adult gland. Furthermore, there is increasing evidence that these stem and early progenitor cells may be targets for transformation during breast carcinogenesis. Normal stem cells and their transformed counterparts share many characteristics, including the ability to under self-renew, to differentiate, albeit abberantly in cancer, telomerase expression, resistance to apoptosis, and ability to metastasize. Thus, we and others have postulated that a key event in transformation may be the disregulation of pathways that regulate normal stem cell self-renewal.

We have described an in vitro system for the propagation of human mammary stem and progenitor cells in suspension culture. We demonstrated that human mammary cells isolated from reduction mammoplasties generates spherical colonies in suspension that we have termed "mammospheres" which are highly enriched in mammary stem and progenitor cells capable of both self-renewal and multi-lineage differentiation.

We have utilized this culture system to investigate the molecular pathways that regulate stem cell self-renewal including Hedgehog, Notch, and Bmi-1. Utilizing real time PCR, we demonstrated that components of both Hedgehog and Notch signaling are highly activated in mammospheres compared to cells cultured under differentiating conditions. We demonstrate bi-directional cross-talk between Hedgehog and Notch signaling, with both signal transduction pathways resulting in the regulation of the polycomb gene Bmi-1. Since Bmi-1 has been found to regulate the self-renewal of normal hematopoietic and neuronal stem cells, we investigated whether the effects of Notch and Hedgehog were mediated by a Bmi-1. Expression of siRNA to Bmi-1 abrogated the stimulatory effects of Hedgehog and Notch signaling on mammosphere formation. In order to further investigate the effects of disregulation of these self-renewal pathways on mammary development, we utilized an in vivo system in which mammospheres are transplanted into the fat pads of NOD-SCID mice, humanized by introduction of irradiated human mammary fibroblasts. We demonstrate that over-expression of the Hedgehog target gene Gli2 in mammosphere initiating cells produces ductal hyperplasia.

We have previously found that breast cancers are driven by a subcomponent of cells which are CD44+ CD24 low lin- that demonstrate stem cell properties. As few as 200 cells with this phenotype are capable of transferring tumors in NOD-SCID mice, whereas 20,000 cells that do not bear this phenotype are nontumorigenic. Furthermore, the tumors generated by these tumor initiating cells, recapitulate the phenotypic heterogeneity of the original tumor as would be predicted from a stem cell model. Tumor stem cells display increased Hedgehog signaling and Bmi-1 expression compared to non-tumorigenic cells isolated from the same tumor. Together these studies support the tumor stem cell hypothesis in which transformation of mammary stem and/or progenitor cells produces mammary tumors driven by a cancer stem cell component. The cancer stem cell model has important implications for developing effective strategies for cancer prevention and treatment.

3 Invited

Local and systemic effects that promote human breast tumorigenesis: insights on heterotype interactions from xenograft models

C. Kuperwasser, M. Wu, D. Proia, J. Weremowicz, A. Richardson, S. Naber. *Tufts University School of Medicine, Molecular Oncology Research Institute (MORI), USA*

The study of normal breast epithelial morphogenesis and carcinogenesis in vivo has largely utilized rodent models. Efforts at studying mammary morphogenesis and cancer with xenotransplanted human epithelial cells have failed to recapitulate the full extent of development seen in the human breast. We have developed an orthotopic xenograft model in which both the stromal and epithelial components of the reconstructed mammary gland are of human origin. Genetic modification of the human stromal cells prior to the implantation of ostensibly normal human mammary epithelial cells resulted in the outgrowth of benign and malignant lesions. We have extended this tissue reconstitution model and combined it with defined genetic manipulation of human breast epithelial tissues to create an in vivo model of human breast cancer in mice. Invasive human breast adenocarcinomas developed as early as 5 weeks post implantation when the expression of dominant oncogenes was combined with a specialized stromal microenvironment. The development of these tumors was dependent on specialized stromal fibroblasts, as tumors rarely developed in the absence of this microenvironment. This experimental model allows for studies of human epithelial morphogenesis and differentiation in vivo and underscores the critical role of heterotypic interactions in human breast development and carcinogenesis.

Wednesday, 22 March 2006 14:15–16:00

SCIENTIFIC SESSION

Specific issues in metastatic disease

4 Invited

Has first-line therapy had an impact on general outcome in metastatic breast cancer?

C. Hudis. *Memorial Sloan Kettering Cancer Center, New York, USA*

Conventionally, clinically detected metastatic growths of breast cancer are not considered curable but are, instead treatable. Usual options include systemic treatment with a sequence of hormonal therapies and manipulations if appropriate, single agent and/or combination chemotherapy regimens alone or combined with newer targeted antibodies (trastuzumab and bevacizumab as indicated), and supportive care. Ultimately, most patients with metastatic breast cancer die of this disease although some have competing causes of mortality.

Despite many clinical trials and a long list of active drugs, proof that first line therapy actually extends life has been historically difficult to obtain. Indeed, support for systemic treatment, and chemotherapy in particular, came from studies suggesting that it improved quality of life even if it did not extend life. Recently, however, several lines of evidence clearly suggest that first line therapy improves general outcomes including overall survival.

Untreated metastatic breast cancer is or can be painful and unpleasant and the immediate beneficial effects of active therapy can be observed in concrete fashion in terms of objective responses in measurable lesions, diminished pain (and use of pain medication), increased performance status, and improved overall quality of life. In many cases tumor response and progression-free survival can serve as surrogates for these endpoints. The challenge, given the sometimes long natural history of metastatic breast cancer and the multiple lines of active therapy available, has been to demonstrate that this evidence of activity in the first-line setting translates to improved overall survival.

In the use of hormone therapy, demonstration of improved overall survival remains challenging as appropriate tumors often have an indolent course, can be treated with multiple active interventions, and occur in older patients for whom other causes of mortality are operative. This was true for the past few decades and remains true today. Yet "general outcomes" are improved by the use of safer and more active drugs (ie, selective aromatase inhibitors) even if overall survival can not be shown to be consistently improved.

For chemotherapy there is evidence from multiple trials suggesting improved survival as well as quality of life with modern drugs and regimens.