26th Pezcoller Symposium: Cancers Driven by Hormones

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Abstract

This symposium was held in Trento, Italy, on June 19–21, 2014, and was focused on advances in biology, physiology, and pathology of neoplasms affected by hormones, especially breast and prostate cancers. The stem cell function, the genetic and epigenetic interactions with hormones, the mechanisms of estrogen receptor transcription, biochemical markers and therapeutic targets in breast cancer, promotion of breast cancer carcinogenesis by progestosterone, the basis for prostate cancer progression and the relevance of DNA repair processes, androgen receptor programming during prostate carcinogenesis, the metabolic stress role in tumor survival, and the diagnostic use of imaging in prostate cancer were discussed.

Hans Clevers (Utrecht Institute, Utrecht, the Netherlands) gave the keynote address. He defined Lgr51,2 as a Wnt target gene that drives crypts stem cells in colon cancer. Lgr51,2+ stem cells generated all epithelial lineages throughout life. Lgr51,2+ stem cells can initiate ever-expanding crypt–villus organoids in three-dimensional (3D) culture. Intestinal cancer is initiated by Wnt pathway–activating mutations in genes, such as APC. Deletion of APC identifies the stem cell as the cell-of-origin of adenomas. A stem cell/progenitor cell hierarchy is maintained in early stem cell–derived adenomas, lending support to the “cancer stem cell” concept. Lgr5 stem cell division occurs symmetrically. Paneth cells are CD24+, and express EGF, TGF–α, Wnt3, and the Notch ligand Dll4, all essential signals for stem-cell maintenance in culture. Coculturing of stem cells with Paneth cells improves organoid formation. Genetic removal of Paneth cells in vivo results in the concomitant loss of Lgr5 stem cells.

Mechanisms of Tumor Cell Hormone Responsiveness

Sohail Tovazoie (The Rockefeller University, New York, NY) indicated that small RNAs, ApoE, and LRP comprise a druggable antimetastatic melanoma network. Specific microRNA sets govern metastatic progression of breast cancer and melanoma. MiR–199a–5p, miR–199a–3p, and miR–1908 are highly overexpressed in metastatic melanoma cells and are metastatic promoters and conversely target the secreted protein ApoE, which is a strong suppressor of melanoma invasion, endothelial recruitment, and metastatic colonization. ApoE is a secreted protein that mediates these effects through its engagement of the LRP1 receptor on melanoma cells and LRP8 receptor on endothelial cell. Transcriptional ApoE induction (through pharmacologic activation of the Liver-X nuclear hormone receptor) inhibits tumor growth, endothelial cell proliferation, and metastasis.

Hormonal Signaling in Breast Cancer

Myles Brown (Dana Farber Cancer Institute, Boston, MA) outlined the genetic and epigenetic determinations of hormone dependence. Specific drugs targeting the enzymes responsible for steroid synthesis and the steroid receptors and the development of the estrogen receptor (ER) led to the first breast cancer predictive biomarker and the first molecularly defined therapeutic target. ER mutations can explain resistance to endocrine therapy in 20% of patients with advanced disease. These mutations activate the receptor in the absence of hormone and make it resistant to existing antagonists. They support the conclusion that the tumor-initiating cell is ER dependent. Mutations in other components of hormone signaling have not yet been validated as mechanisms of endocrine resistance. Genetic mechanisms of
resistance and potential epigenetic mechanisms involving a change in chromatin state have been explored.

Jason Carroll (Cancer Research UK, Cambridge, UK) examined ER transcription in breast cancer; it rarely associates with promoter regions of target genes and instead associates with enhancer elements at distances from the target genes. Forkhead protein FoxA1 maintains interactions between ER and chromatin. The complex of ER with FoxA1 and GATA3 conditions hormonal responses.

Unexpected interactions occur between the ER complex and PR and have a role for PR as a functional regulator of ER activity. PR mediates DNA associations of ER. PR-negative tumors may not reflect tumors with nonfunctional EPR pathways, but represent tumors with copy number loss of the PR gene, which subsequently affects ER binding and transcriptional activity.

Cathrin Briskien (Ecole Polytechnique Federale Lausanne, Switzerland) indicated that progesterone is a major regulator of cell proliferation and stem cell activation in the adult mammary gland. PR activation of Numb ligand (RANKL) and Wnt4 have distinct roles as downstream mediators of PR signaling. 17β estradiol plus PR activates the canonical Wnt pathway in the ex vivo model of human breast tissue microstructures; 17β estradiol plus PR agonists increases cell proliferation. The ex vivo model of these microstructures showed that major signaling pathways are conserved between mouse and man.

**Translational Developments in Hormone-Driven Breast Cancer**

Rene Bernardes (Nederland Cancer Center, Amsterdam, the Netherlands) described breast cancer molecular diagnostics. The genome takes center stage in key treatment decisions for breast cancer. Measurement of ER was the first diagnostic for a targeted cancer drug. Staining HER2 for the treatment with trastuzumab was also first used in breast cancer. A number of molecular diagnostic assays have been developed to help determine the risk of recurrence in breast cancer, including Mamma Print, OncotypeDx, and Pros Gina.

Carlos Arteaga (Vanderbilt-Ingram Cancer Center, Nashville, TN) discussed the PI3K/AKT/TOR as a therapeutic target in breast cancer. Several drugs targeting the PI3K network have been developed. ATP mimetics that bind competitively and reversibly to the ATP-binding pocket of p110 isoform are in clinical development. These include pan-PI3K inhibitors, p110α-specific inhibitors, p110δ-specific inhibitor CAL-101, and dual PI3K/mTOR inhibitors. The pan-PI3K and p110α-specific inhibitors are equally potent against p110α mutants. In recent phase I trials with dual inhibitors, few responses were observed in patients with and without detectable PI3K pathway mutations. Allosteric ATP-competitive pan-inhibitors of the three isoforms of AKT are being developed. Allosteric inhibitor MK-2206 binds to the AKT PH domain and/or hinge region to promote an inactive conformation of the AKT signaling in vivo, and suppresses growth of breast cancer xenografts harboring PI3KCA mutations or ERBB2 amplification. Phase I data showed that treatment with MK-2206 decreases P-AKT levels, in tumor cells, peripheral blood mononuclear cells, and hair follicles. Another approach to block this pathway has been the development of ATP-competitive inhibitors of the mTOR kinase. Dual PI3K/mTOR inhibitors have also been developed in the hope of overcoming the loss of feedback inhibition or PI3K activation.

Mitch Dowsett (Institute of Cancer Research, London, UK) described biomarkers of response and resistance to estrogen deprivation in breast cancer. Multiple metagenes representing proliferation and estrogen-responsive pathways were profoundly reduced by aromastase inhibitors, as were signaling pathways, potentially contributing to benefit from therapy. In contrast, expression of certain genes was significantly increased by treatment, suggesting that the resistance-relevant pathways are upregulated at early time point.

In ER-positive breast cancers treated for 5 years with tamoxifen, one can identify early recurrence at 5 years versus late recurrence at 10 years. A total of 1,327 genes predicted early versus late recurrence.

Pier Paolo Di Fiore (IFOM-IEO Campus, Milan, Italy) discussed one mechanism of coupling cell-fate determinations and tumor suppression in mammary stem cells. Numb is a protein that, by asymmetrically partitioning at mitosis, controls binary cell-fate decisions. In human breast cancers, there is frequent loss of Numb expression. The lack of Numb allows for unchecked signaling activity of the Notch receptor and causes attenuation of the p53 signaling pathway. This latter function is exerted through Numb-mediated inhibition of the ubiquitinating function of the E3 ligase Mdm2 over p53. Thus, loss of Numb allows for the simultaneous activation of an oncogene (Notch) and attenuation of an oncosuppressor (p53), in a circuitry shown to be relevant to the subversion of homeostasis in the mammary stem cell compartment.

**Hormonal Signaling in Prostate Cancer**

Arul M. Chinnaiyan (Michigan Center for Translational Pathology, Ann Arbor, MI) discussed progress toward precision medicine for advanced prostate cancer. MI-ONCOSEQ is a unique clinical sequencing program, which consists of three integrated projects: (i) "Clinical Genomic Study"; (ii) "Sequencing and Analysis" progresses, biospecimens with sequencing and analysis of tumors for point mutations, copy number changes, gene fusions and aberrant gene expression; (iii) "Ethics and Psychosocial Analysis." More than 250 adults and 40 children with cancer have been sequenced with return of results through a precision medicine tumor board (PMTB). Discoveries include the identification of a novel recurrent NAB2-STAT6 gene fusion in solitary fibrous tumors, a rare soft-tissue tumor rearrangement involving targetable FGFR in various cancers and identification of ESR1 mutations acquired after antiestrogen therapies. It was found that protein BRD 4 activates Myc and is a therapeutic target in prostate cancer.

Owen Witte (University of California, Los Angeles, Los Angeles CA) discussed multiple pathways that can be involved in prostate cancer progression to castration-resistance stage. Mouse and human benign prostate epithelial basal stem cells have been defined as one cell of origin that can respond to multiple stimuli to produce cancers, which are capable of maturing to more differentiated cell types, including luminal, squamous, and neuroendocrine phenotypes. Current work is focusing on the activation of specific kinase-driven pathways to provide new therapy targets in metastatic prostate cancer. Tyrosine phosphorylation is increased in castration-resistant prostate cancer despite low androgen receptor (AR) mutations.

Antonella Fanetti (Ist. Naz. Tumori Regina Elena, Rome) discussed estrogen/ER, hypoxia/hypoxia-inducible factor (HIF), and NO/eNOS signaling in hormone-driven cancers. Nuclearized
endothelial NOS (eNOS) are partners of both ER and HIFs in prostate cancer. A number of eNOS–DNA associations exist that define transcriptional active regions modulated by estrogen. eNOS participates in the regulation of large gene sets, including noncoding RNAs (miRNA). A cluster of miRNAs are silenced in prostate cancer cells. A feedback loop was identified involving transcriptional downregulation of pri-miR-34a by the eNOS/SIRT1 complex in an estrogen-dependent fashion. Induction of the miR-34a target SIRT1 (an epigenetic regulator of aging and tumorigenesis) sequentially activates eNOS itself by posttranslational modification. These findings revealed novel functions of eNOS and of the eNOS/SIRT1 interplay, fine-tuned by E2-activated ER signaling, favoring the concept of eNOS as a critical molecular determinant in aggressive prostate cancer. A number of eNOS-bound complexes in the genome regions of many IncRNAs strictly associated with cancer have been identified. Preliminary data indicate that (i) IncRNA HOTAIR and H19 basal expression in breast cancer cell lines appears highly correlated with presence of ERα, being undetectable in the ERα− cells; (ii) IncRNA HOTAIR, MALAT1, and GAS5 have higher expression levels in metastatic prostate cancer cell lines than in primary tumor-derived prostate cancer cells; (iii) IncRNA H19, HOTAIR, and CDKN2B-AS1/ANRIL expression significantly decreases after eNOS inhibition exclusively in ERα− breast cancer cell lines, strongly suggesting that eNOS and ERα are strictly required for the transcriptional control of selected cancer-associated IncRNAs in hormone-driven cancers.

Roland Schuël (University of Freiburg Medical Center, Freiburg, Germany) discussed the physiopathology of the epigenetic histone lysine-specific demethylase LSD1, which is associated with AR in prostate and prostate cancer. Epigenetic control mechanisms serve as an additional layer in gene expression regulation. LSD1 removes mono- and dimethyl groups from H3K4 or H3K9, thereby causing either repression or activation of gene transcription. The roles of LSD1 in regulating chromatin organization and AR-dependent gene expression in prostate cells have been uncovered. The chromatin remodeler CHD1 binds to methylated histone and interacts with methylated LSD1, blocking ligand-induced AR recruitment.

Matthew Freedman (Dana Farber Cancer Institute, Boston, MA) outlined charting the AR cistrome in human prostate tissue. The genome-wide set of AR-binding sites was mapped in 21 normal and prostate cancer samples using chromatin immunoprecipitation followed by high-throughput sequencing: It was established that HOXB13 and FOXA1 reprogram the AR cistrome during prostate tumorigenesis. The identification of differential AR-binding sites between normal and tumor revealed key AR coregulators as well as AR target genes that distinguish indolent from aggressive prostate cancer.

Karen Knudsen (Thomas Jefferson University, Philadelphia, PA) discussed AR–DNA repair interplay in prostate cancer and their implications for disease progression. Alterations in DNA damage repair (DDR) pathways are common in sporadic prostate cancer, and alterations in these pathways may accord effective means of therapeutic intervention. First, alterations in genes whose functions are key for DNA repair increase in frequency as a disease-progression function. Second, the AR is a critical effector of DNA repair competence that alters the response to genotoxic insult in advanced prostate cancer. AR regulates the expression and activity of DNAPK, an enzyme that is key for repairing double-strand DNA breaks, and a transcriptional modulator. Third, clinical trials revealed that therapeutic agents that target the DDR pathway are effective in treating advanced prostate cancer, consistent with the postulate that a subset of advanced tumors has altered DDR programs.

Translational Developments in Hormone-Driven Prostate Cancer

William Hait (New Brunswick, NJ) discussed the metabolic stress relevance in cancer. Nutrient and growth factor deprivation, hypoxia, and low pH create metabolic demands that require cellular adaptations to sustain energy levels. Protein synthesis is a most notable energy consumer. Mounting evidence implicates control of protein synthesis as a survival mechanism for both normal and malignant cells. Autophagy conserves energy. Ca/Ca2+/Calmodulin-dependent kinase activity differs in normal versus cancer cells; elongation factor-2 kinase, a downstream component of the PI3K/AKT pathway that behaves as a critical checkpoint in energy consumption, is phosphorylated only in tumors in the presence of Ca/Ca2+/Calmodulin and it inhibits protein translation. This kinase is inhibited by activated MTOR and S6K; its knockdown causes a decrease of autophagy. Starvation induces its activation. It regulates increases in peptide elongation in starvation. In prostate cancer, responses to enzobutamide are increased by a decrease of autophagy. Abiraterone acetate is effective in castration-resistant prostate cancer (CRPC).

Srinivasan Yesunatherammian (The Johns Hopkins University, Baltimore, MD) discussed androgen-induced double-stand breaks (DSB) in prostate cancer. Induction of AR-mediated transcriptional programs by androgen stimulation also involves DNA damage, DSB, and DSB repair proteins. The DSB can be mediated by topoisomerase TOP2B, which is recruited with AR to regulatory sites on target genes and is required for efficient transcriptional activation of these genes. If illegitimately repaired, such DSBs can contribute to cancer progression by promoting genetic instability and seeding formation of genomic rearrangements, such as the recurrent TMPRSS2–ERG fusion oncogene in prostate cancer. These androgen-induced TOP2B-mediated DSBs may be exploitable therapeutically. Short pulses of androgen stimulating in the backdrop of androgen depletion therapy could selectively sensitize prostate cancer cells to DNA damaging agents or to DNA repair inhibitors.

Robert Reiter (University of California, Los Angeles) outlined the usage of PET and optical imaging of prostate cancer with engineered antibodies. The utility of engineered antibody fragments targeting major prostate cancer antigens, such as prostate stem cell antigen (PSCA) and prostate membrane antigen (PSMA), to image prostate cancer was explored. Engineered antibody fragments retain the specificity of intact antibodies while providing for tunable clearance, reduce serum half-life and potentially improved tumor penetration. Engineered PSCA minibodies and diabodies for both optical and PET imaging of prostate, bladder, and pancreatic cancers were developed, as was a PSMA-targeted minibody for imaging advanced prostate cancer. The use of optical fluorescent PSCA probes for intraoperative visualization of prostate cancer to eradicate more completely tumors was illustrated. The preclinical and early clinical development of PSCA and PSMA minibodies for PET imaging of high-risk and metastatic prostate cancer offers the potential for more sensitive and specific staging of disease and for setting up therapies targeting these antigens.
Martin Gleave (Vancouver Prostate Center, Vancouver, Canada) discussed co-targeting the AR and the adaptive stress pathway in CRPC. Molecular chaperons mediate stress responses by regulating autophagy and transcriptional survival networks. Two stress-activated cytoprotective chaperones, clustering (CLU) and Hsp27, are targets in clinical trials of CRPC.

CLU is transcriptionally regulated by stress-associated HSF1 and YB-1, retrotranslocating from the ER to cytosol to inhibit apoptosis by suppressing protein aggregation, p53-activating stress signals, and Bax, while enhancing Akt phosphorylation and transactivation of NF-κB, YB-1, and HSF-1. CLU supports tumor cell survival under stress conditions by facilitating autophagosome biogenesis. CLU confers treatment resistance in cancer, whereas CLU inhibition potentiates anticancer therapies, including enzobutamide, in preclinical models. The antisense oligonucleotide OGX.011 inhibits CLU and is in phase III trials of CRPC and lung cancer in combination with Taxol. Hsp27 expression is induced by hormone and chemotherapy and inhibits treatment-induced apoptosis. Hsp27 inhibition may simultaneously suppress many pathways implicated in cancer progression and resistance to hormone- and chemotherapies. In a randomized phase II study of the Hsp27 inhibitor OGX-427 in patients with CRPC, preliminary results indicate PSA decline and freedom from progression at 12 weeks compared with prednisone controls. These results confirm, for the first time, single-agent activity for an Hsp27 inhibitor in cancer.

Summary

Hormones affect the incidence, natural history, and clinical outcome of common cancers. Clinical care of these tumors should be based upon a knowledge of molecular events that underlie biochemical, cell biological, and pathophysiological phenomena characteristic of each tumor in each patient. This symposium explored advances arising from a knowledge of the mechanisms of these phenomena. The major emphasis was on breast and prostate cancers.

In both tumor types, the response to hormones and hormone inhibitors was shown to be dependent on specific genes and their expression; gene mutations, chromatin state, and signaling pathways are all relevant in this respect.

Epigenetic regulation of gene expression and the epigenetic characterization of tumors are important. Knowledge of the molecular mechanisms of implementation of the genetic and epigenetic processes provides a basis for the development of new therapies that may be effective in specific patients or in a restricted group of patients. Indeed, the genome and related mutations take increasing importance in the treatment design for many tumors, including breast and prostate neoplasms.

Information discussed at the meeting supported the concept of cancer stem cells; clarification of phenomena involved in determining the decision of these cells about their fate provides leads for the development of new therapies. The utilization of engineered mini antibodies against stem cell antigens can improve the identification of these cells by fluorescence and also indicates the possibility of designing specific anti–stem cell treatments.

A functional cooperation between ER and HIF is a new area to be exploited for therapeutic intervention.

Tumor survival mechanisms in the face of metabolic stress must include consideration of the fact that protein synthesis is using much energy and that autophagy conserves energy. Mechanisms causing energy consumption and affecting tumor growth should be useful sites for the design of new therapies.

Altered DDR enzymes may also represent sites for therapeutic intervention; patients should be identified with altered DNA repair for suitable personalized treatments.

In conclusion, this symposium gave an integrated view of the role of a number of molecular factors and pathways in determining hormonal effects on the growth, and the growth inhibition by, and resistance to, inhibition of hormone function, of breast and prostate cancers; many of the phenomena discussed are of relevance to other types of tumors. Indeed, the meeting provided many promising leads for the development of new therapies and the achievement of the so-called personalized or precision medicine.

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