Modeling Human Cancer: Report on the Eighth Beatson International Cancer Conference

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Abstract

One of the major challenges facing cancer researchers is the necessity to adequately model the human disease. This need is driven by goals, including validating/determining the contribution of a gene to cancer, establishing systems for drug discovery and validation, and discovering additional factors that modify the oncogenic process. Recent advances in cancer modeling were discussed at the Eighth Beatson International Cancer Conference on June 19 to 22, 2005 in Glasgow, United Kingdom. The conference was attended by 250 scientists with >130 oral and poster presentations. The research presented made use of systems ranging from two-dimensional and three-dimensional cell culture to nonmammalian and mammalian *in vivo* models. In addition to basic research on the fundamental processes involved in cancer, novel developments in cancer treatment were presented. (Cancer Res 2005; 65(24): 11247-50)

Introduction

Model systems are, by definition, being used to study human cancer unless research is done directly on human subjects or using clinically derived material. Immortal tumor cell lines have been invaluable tools of modern cancer research; however, more sophisticated models are needed to drive progress forward. The Eighth Beatson International Cancer Conference, entitled "Human Cancer: Modelling the Disease," brought together scientists working on a variety of cancer models. These included genetic systems designed to identify and examine modifiers of tumorigenesis, *in vivo* cancer models to investigate interactions between oncogenes and/or tumor suppressors and to validate targets for anticancer therapies, and three-dimensional *in vitro* cell culture to more accurately mimic the *in vivo* microenvironment with a particular focus on tumor cell invasion and metastasis.

Keynote Lecture

The conference began with a presentation by Douglas Hanahan (University of California, San Francisco, CA) on the "angiogenic switch," which is triggered by the demands of tumor hyperproliferation. Tumor angiogenesis involves more than cancer cells acting on vascular endothelial cells. Infiltrating leucocytes are attracted to regions of neoplastic growth and release factors, including matrix metalloproteases (MMP), cathepsins, and heparanase, which liberate sequestered vascular endothelial growth factor (VEGF). Although genetic deletion of *VEGF-A* or *MMP9* in mice reduced tumor growth, targeting VEGF receptor function has produced limited clinical effects against end-stage tumors, and positive effects on earlier-stage tumors may be transient. Pericytes contribute to the stability and maintenance of blood vessels and are regulated by paracrine platelet-derived growth factor (PDGF) signaling from endothelial cells. These findings suggest that PDGF receptor blockade, which disrupts pericyte coverage and destabilizes tumor (but not normal) vasculature, in combination with VEGF receptor inhibition would result in more efficacious and long-lived therapy.

Cell Invasion and Metastasis

Cell motility and migration have largely been studied in tissue culture dishes. However, the physiologic relevance of these studies has been challenged because of the significant differences between cell motility in two dimensions versus three dimensions, as well as differences between rigid versus compliant substrates. To overcome these shortcomings, three-dimensional artificial matrices and *in vivo* models are increasingly being used to examine tumor cell invasiveness.

Two forms of tumor cell invasion have been defined: a mesenchymal mode dependent on extracellular matrix proteolysis and a proteolysis-independent amoeboid mode. These two modes may reflect underlying genetic differences that could have significant clinical implications. Erik Sahai (London Research Institute, London, United Kingdom) has imaged MTLn3 breast carcinoma cells invading collagen via the amoeboid mode, which is sensitive to inhibition of the serine/threonine kinase ROCK. Active ROCK increases type II myosin light-chain phosphorylation; myosin light chain is bundled in leading protrusions perpendicular to the direction of movement. As cells moved, collagen fibers were deformed but not degraded. *In vivo* (monitored by two-photon microscopy) myosin light-chain distribution was similar to that seen in amoeboid motility, and collagen was deformed but not degraded. Therefore, amoeboid motility requires significant force generation through actin-myosin contraction to deform the extracellular matrix and allow cells to push through without proteolysis, whereas mesenchymal motility has a small requirement for force generation but is dependent on extracellular matrix proteolysis.

The effect of blocking extracellular proteases on tumor cell invasion in three-dimensional matrices was presented by Neil Carragher (AstraZeneca, Loughborough, United Kingdom). Not only is amoeboid motility insensitive to protease inhibitors, some cells may also undergo a "mesenchymal to amoeboid" transition that allows invasion without matrix degradation. Focal adhesion turnover was not required as inhibition of calpain2 protease or Src had no effect. When ROCK activity was blocked, tumor cells underwent an "amoeboid to mesenchymal" transition to calpain2-dependent invasion. Amoeboid invasion is associated with weak integrin-mediated adhesion, possibly due to lowered N-linked

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glycosylation of $\alpha_2\beta_1$ integrin, eliminating the need for calpain2-mediated focal adhesion turnover. The combination of ROCK and calpain inhibition was more effective than either alone at reducing invasion.

Factors in the tumor microenvironment might trigger the mesenchymal to amoeboid or reciprocal transition. Emmanuel Vial (Institute of Signalling Developmental Biology and Cancer, Nice, France) reported on the HECT domain E3 ubiquitin ligase Smurf1, which induces RhoA degradation. When Smurf1 was knocked down, there was localized RhoA accumulation, changed cell morphology, and decreased two-dimensional motility. In three dimensions, Smurf1 knockdown lead to amoeboid migration, while Smurf1 knockdown in tumor xenografts produced similar results, suggesting that Smurf1 is an important regulator of the mesenchymal/amoeboid decision.

ROCK appears to be critical for amoeboid motility *in vitro*. To test whether ROCK activation promotes tumor cell invasiveness *in vivo*, Michael Olson (Beatson Institute, Glasgow, United Kingdom) fused the ROCK II kinase domain to the estrogen receptor hormone binding domain to generate conditionally active ROCK:ER. Activation of ROCK:ER resulted in cell adhesion protein relocalization and increased cell motility *in vitro*. ROCK:ER activation in s.c. xenograft tumors resulted in cell spreading into surrounding tissue and increased tumor vascularization. Therefore, ROCK activation leads to increased tumor cell invasiveness and increased angiogenesis, possibly because increased tumor tissue plasticity allows endothelial cells to more readily access hypoxic regions.

Genetic Validation of Cancer Targets

A major question in cancer research is whether a given protein is a potential therapeutic target. One method to answer this question is conditional gene deletion in established genetically modified cancer models.

Genetically modified colorectal cancer models were presented by Alan Clarke (Cardiff University, Cardiff, United Kingdom). The *LKB1* tumor suppressor is mutated in Peutz-Jeghars syndrome, which is characterized by multiple gastrointestinal hamartomatous polyposis. Long-term *LKB1* deletion revealed that the epithelial cell lineage is *LKB1* dependent; otherwise, cells default to the secretory lineage. *LKB1* deletion results in a low frequency of intestinal hamartomas, a tumor type characterized by numerous differentiated epithelial cells. Two genes associated with neoplasias induced by loss of *APC* function were also examined; deletion of either methyl-CpG binding protein 2 (*Mbd2*) or secreted protein acid rich in cysteine (*SPARC*) attenuated tumor formation. The reduction in tumor formation associated with *SPARC* deletion may have important clinical relevance given the positive associations among elevated SPARC levels, tumor progression, and patient prognosis. Owen Sansom (Beatson Institute) also used genetic deletions to analyze the oncogenic program initiated by *APC* disruption. Long-term *c-Myc* deletion led to loss of stem cells, indicating that *c-Myc* is required for maintenance of *APC*-deficient cells. These results suggest that there is therapeutic potential for c-Myc inhibition in colorectal cancer.

Conditional *c-Myc* deletion was also used by Andreas Trumpp (Swiss Institute for Experimental Cancer Research, Epalinges, Switzerland) to examine its role in development and oncogenesis. Deletion of *c-Myc* in the mouse embryo, with continued placental expression, allowed development further than the early lethality observed following total *c-Myc* deletion, suggesting that after the production of stem cells and/or transit amplifying cells, c-Myc may be dispensable for subsequent cell divisions that lead to tissue formation. Deletion of *c-Myc* in Keratin 5–expressing epidermal cells did not affect skin development nor did it attenuate phorbol ester–induced hyperproliferation. However, *c-Myc* deletion did reduce papilloma formation in a chemical carcinogenesis skin cancer model, suggesting that c-Myc may be a therapeutic target for additional cancers, including squamous cell carcinomas.

c-Myc collaboration in oncogenesis was also presented by Karen Blyth (University of Glasgow, Glasgow, United Kingdom). Crossing mice expressing *Runx2* regulated by the T cell–selective CD2 promoter with mice expressing CD2 regulated *c-Myc* resulted in accelerated T-cell lymphoma development. The collaboration between Runx2 and c-Myc seems to work reciprocally; c-Myc counteracts a Runx2-induced proliferation defect, whereas Runx2 attenuates c-Myc-induced apoptosis. Crossing CD2-*Runx2* mice with Eμ-*Pim1* mice also accelerated tumor development. These results reiterate the importance of *c-Myc* and suggest that Pim serine/threonine kinases may be critical parts of a multicomponent oncogenic program.

One therapeutic avenue evaluated using genetically modified mouse models was cell cycle inhibition. Given that genetic changes to many cell cycle regulators [cyclin-dependent kinases (CDK), their activating cyclin partners, CDK inhibitors (CDKIs), and downstream substrates including retinoblastoma (Rb)] have been associated with cancer, CDK activity has long been assumed a valid target. The laboratory of Mariano Barbacid (Centro Nacional de Investigaciones Oncógicas, Madrid, Spain) has used gene targeting to delete *CDK2, CDK4*, and *CDK6* to validate them as potential targets. *CDK2* deletion in chemically induced skin papillomas did not alter tumor development, indicating that CDK2 does not contribute to this cancer. In addition, *CDK2* deletion in $p27^{Kip1}$ null mice did not affect pituitary tumor development or reduce organomegaly and overall increased body size. Philipp Kaldis (National Cancer Institute, Frederick, MD) reported similar results: although his laboratory found that pituitary and ovarian tumors in $p27^{Kip1}$ null mice were not affected by *CDK2* deletion, the increased body size was reverted in male mice. *CDK2* deletion resulted in increased CDC2 (CDK1) activity, whereas RNA interference–mediated CDC2 knockdown reduced $CDK2^{-/-}$ cell proliferation more than in wild-type cells. These results suggest that in the context of *CDK2* deletion, other CDKs, such as CDC2, may compensate. However, the influence of $p27^{Kip1}$ may act through CDKs other than CDK2. Therefore, CDK2 inhibition alone may not be an effective therapy, although it remains to be seen whether human tumors behave similarly. These results also suggest anticancer pharmacologic inhibitors that are not strictly specific for CDK2, CDK4, or CDK6 but also capable of simultaneous inhibition of CDC2/CDK1 may be more efficacious.

Oncogenes and Tumor Suppressors

Nucleophosmin (NPM) may act as an oncogene or a tumor suppressor, as discussed by Pier Paolo Pandolfi (Memorial Sloan Kettering Cancer Center, New York, NY). *NPM* deletion results in embryonic lethality at E10.5; primary cells and tissues have elevated p53 levels, increased apoptosis, aberrant mitotic spindles with centrosome amplification, and polyploidy. These effects may reflect a role NPM in regulating centrosome number. Consistent with a tumor suppressor function, *NPM* null cells are more readily

transformed by c-Myc or adenovirus E1A protein, whereas *NPM* heterozygosity cooperates with Eμ-*Myc* to induce B-cell lymphomas in mice. Consistent with these genetically modified mouse results, the *NPM* locus is deleted or mutated in some human cancers, such as myelodysplastic syndrome. *NPM* heterozygous mice develop hematologic features that resemble human myelodysplastic syndrome. Despite acting as a tumor suppressor, *NPM* also has oncogenic properties when overexpressed, possibly by sequestering $p19^{ARF}$. Therefore, *NPM* gene dosage determines whether it acts as an oncogene or tumor suppressor.

The *erbB-2* oncogene is activated in numerous epithelial cancers, notably in breast cancer. To model the processes involved in human cancer, William Muller (McGill University, Montreal, Quebec, Canada) derived genetically modified mice with activated *erbB-2* regulated by the endogenous *erbB-2* promoter. When induced in mammary glands, one to two nonmetastatic tumors developed per mouse ~16 months later, with 90% penetrance after 2 years. Tumor progression was associated with increased erbB-2 expression and genomic amplification of the *erbB-2* allele. β_1 *integrin* deletion reduced mammary tumor induction in MMTV-polyoma middle T expressing mice that rapidly develop metastatic tumors.

The role of the activator protein (AP-1) dimeric transcription factor complex (the best known constituents being Fos and Jun proteins) in cancer was the subject of the presentation by Erwin Wagner (Research Institute of Molecular Pathology, Vienna, Austria). Overexpression of *c-Fos* in genetically modified mice led to osteosarcoma formation, whereas *c-Fos* deletion resulted in osteoclast deficiency. When *c-Fos* and *p53* null mice were crossed, skeletal muscle tumors developed. Therefore, *c-Fos* has both oncogene and tumor suppressor properties, depending on the cellular context. The Jun family member c-Jun was found to be required for early stage liver cancer cell survival. Combined *c-Jun* and *Jun B* deletion in the skin of adult mice caused a psoriasis-like phenotype, strongly resembling the human disease. *Jun B* deletion in postnatal mice resulted in myeloid hyperproliferation and splenomegaly. The studies indicate that each component of the AP-1 transcription factor has distinct and tissue selective functions.

Although CDKIs are not mutated in human cancers, they are haploinsufficient for tumor suppression. Martine Roussel (St. Jude Children's Research Hospital, Memphis, TN) examined the contribution of *CDKI* loss to central nervous system cancers. When mice lacking the CDKI $p18^{INK4C}$ were crossed with *p53* null mice, medulloblastomas occurred after 2 to 5 months with low penetrance, whereas no tumors were found in $p18^{INK4C}/p19^{ARF}$ double null mice. If $p18^{INK4C}/p53$ double knockout mice were irradiated 5 days after birth, ~80% developed tumors. When $p18^{INK4C}$ null mice were bred with *Patched* heterozygous mice, double heterozygous animals rapidly developed medulloblastomas, without affecting their *p53* status. The $p18^{INK4C}$/*Patched* double knockout mouse is the first high penetrance mouse model for medulloblastoma without *p53* alterations.

Pancreatic ductal adenocarcinoma (PDA) is one of the deadliest human cancers. Although numerous genetic alterations have been associated with PDA, the precise genetic basis of PDA has not been established. David Tuveson (University of Pennsylvania, Philadelphia, PA) conditionally expressed an active *K-Ras* allele from the endogenous locus in the pancreas of genetically modified mice, resulting in pancreatic intraepithelial neoplasias, indistinguishable from those seen in the human disease, which may represent a premalignant precursor stage to PDA. With time, *K-Ras* expressing mice progressed to fully metastatic PDA, with secondary tumors found in the same tissues as in human cancer, and eventual mortality. When oncogenic *K-Ras* was coexpressed with mutant *p53*, tumors developed more rapidly resulting in earlier mortality.

Genetic Models for Identifying Cancer Genes

In addition to genetically modified mice, nonmammalian *in vivo* and mammalian *in vitro* model systems have been used to examine cancer's genetic basis.

S-phase cell cycle entry is regulated by the Rb "pocket protein" family and their associated E2F and DP transcription regulators. Nicholas Dyson (Massachusetts General Hospital, Charlestown, MA) has used *Drosophila* as a model system to study these proteins because of its simplicity; that is, *Drosophila* has only two Rb, one DP, and two E2F proteins. As in mammals, transcription can be activated (by dE2F1, which associates with RbF1) or repressed (by dE2F2, which associates with RbF1 or RbF2). Deletion of *dE2F1* results in decreased proliferation, whereas *dE2F2* mutants show a slight reduction in viability and fertility. Crossing *dE2F1* and *dE2F2* mutants restored cell proliferation, indicating that neither was necessary for proliferation, and that loss of *dE2F2* removed the growth suppressive effect of *dE2F1* loss.

Drosophila was also used to identify genetic modifiers of organ growth by Nicolas Tapon (London Research Institute). Perturbations in the fly eye of either positive (increased cell proliferation or size) or negative factors (cell death) may be detected. Loss of *Salvador* (Sav) leads to increased cell proliferation, reduced apoptosis associated with increased expression of the antiapoptotic protein DIAP1, and eye overgrowths. Sav protein is a scaffold that contains a coiled coil and two WW domains. The Lats tumor suppressor (known as Warts in *Drosophila*) binds Sav as does Hippo (equivalent to mammalian MST1 or MST2 serine/threonine kinases). Hippo (a proapoptotic kinase) knockdown by siRNA increased DIAP1, whereas *Sav* or *Hippo* elevation reduced DIAP1 protein stability. Given these results and the incidence of *Sav* mutations in human cancers, *Sav* and *Hippo* may be tumor suppressors in a common pathway.

Using *Drosophila* to examine cell and organ growth also is the approach of Tian Xu (Yale University, New Haven, CT). The Lats tumor suppressor may act by binding and inhibiting the CDKs CDC2 and CDK2 and LIM kinase 1, which regulates actin structures. The TSC1/TSC2 tumor suppressor complex regulates Rheb GTPase activity and ultimately S6 kinase activity; TSC complex mutations affect cell growth and size and consequently organ/tissue size. To screen for genetic factors that promote metastasis, benign tumors were induced in fly eyes by expressing oncogenic *Ras*, and then distal tumors were isolated and genotyped. Mutations that affected apical/basal cell polarity lead to metastatic behavior, independent of tumor size. c-Jun NH_2-terminal kinase (JNK) signaling was activated in metastatic tumor cells, and blocking signaling with dominant-negative JNK reduced metastasis.

The strategy of establishing cells with defined genetic changes to screen for additional factors that promote oncogenesis was presented by Reuvan Agami (Netherlands Cancer Institute, Amsterdam, the Netherlands) who developed a plasmid-based method of stably expressing short hairpin interfering RNAs (shRNA) to knockdown target gene expression. Primary human cells were immortalized with telomerase, Ras, and advenoviral large T and small t proteins, then a shRNA library was screened for loss of targets that would permit anchorage-independent growth. One

gene identified was the transcription factor *PITX1*, which regulates expression of RASAL1, a Ras-GTPase-activating protein. These results suggested that PITX1 knockdown lowers expression of RASAL1, which in turn leads reduced GTP hydrolysis on Ras protein(s). A similar screening method has been used to identify micro RNAs that overcome Ras-induced senescence. Therefore, this type of system will be useful for identifying additional genetic modifiers for a variety of oncogenes and tumor suppressors.

Breast cancer cells (MCF10A) grown in three dimensions to more closely mimic the *in vivo* situation was the model system used by Joan Brugge (Harvard, Boston, MA) to probe the biological activities of candidate oncogenes. When cultured in three dimensions, MCF10A cells develop into acini with apicobasal polarity, cease proliferating, and form an interior lumen space as a result of caspase-mediated apoptosis of inner cells. Using this system, the ability of genes to reduce the clearance of the inner cells, by affecting apoptosis and/or proliferation, was monitored. The three-dimensional culture system was also used to identify genes that converted *erbB-2*-induced structures from noninvasive to invasive.

Translational Research

Hans Clevers (Netherlands Institute for Developmental Biology, Utrecht, the Netherlands) presented results from recent studies on colorectal cancer models. Crypt cell proliferation in the small intestine epithelium is dependent on the Notch signaling pathway. When Notch protein processing was blocked with a γ-secretase inhibitor, crypt cells stopped proliferating. In genetically modified mice mutated for the *APC* gene, which spontaneously develop intestinal neoplasias, γ-secretase inhibition blocked the proliferation of cells within adenomas. Therefore, targeting Notch processing with γ-secretase inhibitors may be an effective therapy for colorectal cancer.

George Prendergast (Lankenau Institute for Medical Research, Wynnewood, PA) presented new findings on the cancer genetics underlying immune escape, a process by which tumors evade elimination by the immune system. *Bin1* (a BAR adapter protein attenuated in various cancers) deletion led to immune escape of oncogenically transformed mouse cells via elevation of the catabolic enzyme indoleamine 2,3-dioxygenase (IDO). IDO facilitates immune escape by depleting the local microenvironment of tryptophan, which is essential for antigen activation of T lymphocytes. IDO inhibition cooperated with cytotoxic agents to elicit T cell–dependent regression of established tumors insensitive to single-agent chemotherapy. Therefore, IDO inhibitors may prove valuable for their ability to improve responses to standard cancer chemotherapies by reducing tumor cell immune escape.

In addition to genetically modified mouse cancer models, tumor cell lines continue to have significant value for exploring therapeutic strategies. Alan Ashworth (Breakthrough Breast Cancer Research Centre, London, United Kingdom) presented results from studies on cell lines deleted for the *BRCA* tumor suppressor. These cells have DNA repair defects and were found to be significantly more sensitive to the induction of cell death by DNA cross-linking agents or by inhibitors of poly(ADP-ribose) polymerase (PARP). Based on these results, clinical trials are being initiated to examine the effectiveness of carboplatin or PARP inhibitors on *BRCA1* and *BRCA2* mutant breast cancers.

DNA Replication and Repair Pathways

Ron Laskey (Hutchinson/Medical Research Council Research Centre, Cambridge, United Kingdom) presented data on the importance of MCM3AP and geminin in the regulation of DNA replication. Furthermore, he showed that MCM3AP levels could be stimulated with cytokine treatment in established cancer cell lines but not in primary nontransformed cells, and that this correlated with abnormal DNA replication and cell death only in the transformed cell lines. Jiri Bartek (Danish Cancer Society, Copenhagen, Denmark) reviewed the distinct manners of DNA damage checkpoint activation through the ATR-CHK1 and ATM-CHK2 pathways and noted that the former pathway is required for viability, whereas the latter is not. This may reflect the additional important roles of CHK1 in regulating CDK1/cyclin B and centrosome function in cells, which may have important implications in the development of CHK1 inhibitors. Stephen West (London Research Institute) reported on the important role of the BRCA2 complex in homologous recombination-mediated double-stranded break repair and the involvement of the BRCA2 paralogues in the resolution of Holliday junction intermediates. Ian Hickson (University of Oxford, Oxford, United Kingdom) showed that the Blooms syndrome tumor suppressor also participates in the resolution of Holliday junction intermediates and how that might contribute to the increased rate of sister chromatid exchange and carcinogenesis.

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