

## ■ ANNOUNCEMENT

**ANNOUNCEMENT: New CCR Publication**

**F**rontiers in Science as a stand-alone publication will be coming to an end in June 2007. CCR's intramural scientific news currently covered in *Frontiers* will be integrated into a new publication called *CCR Connections*, which will broaden communications to include external audiences as well. The new publication will highlight CCR's connectivity, its scientific interactions within and outside NCI's Intramural Research Program.

## ■ IN REMEMBRANCE

**In Remembrance: Robert C. Moschel, PhD**

**T**he CCR research community mourns the loss of Robert C. Moschel, PhD, who died April 20 due to complications from pancreatic cancer. Bob, who was both an outstanding chemist and a wonderful friend and colleague, is remembered as a kind and generous man whose work has led to the development of new drugs that enhance the effectiveness of chemotherapy for brain tumors and that could potentially help treat other cancers as well.

Dr. Moschel was born and raised in Cincinnati, Ohio. After receiving his PhD in biochemistry from Ohio State University in 1973, he conducted postdoctoral research in organic chemistry at the University of Illinois for three years and then settled in Frederick, Maryland. Dr. Moschel was promoted to staff scientist in 1987 in the ABL-Basic Research Program and then became head of the Carcinogen-Modified Nucleic Acid Chemistry Section in 1992. His section became part of the CCR at the NCI-Frederick in 1999.

Dr. Moschel and his colleagues developed compounds that can inactivate the human DNA repair protein, *O*<sup>6</sup>-alkylguanine-DNA alkyltransferase (AGT). This protein is primarily responsible for the resistance to chemotherapy that tumor cells exhibit when treated with drugs that act through alkylation of DNA at the *O*<sup>6</sup> position of guanine. The inactivation of AGT can bring about a dramatic improvement in the effectiveness of these drugs. *O*<sup>6</sup>-benzylguanine, one of the alkyltransferase-inactivating drugs developed by Dr. Moschel's lab, is currently in phase I and II clinical trials in combination with



1,3-bis(2-chloroethyl)-1-nitrosourea (BCNU) or temozolomide. His research team continues to develop new inactivators that are more potent, more water soluble, and more selective for tumor cells. These new drugs could prove to be very useful as chemotherapy adjuvants.

Dr. Gary Pauly, who worked with Dr. Moschel for more than 20 years, recalls that “Bob was unstoppable in his determination to bring his compounds from the bench to the bedside. He carried his drugs from discovery to proof of principle, through the pharmacology and toxicology, to the patients in the clinic.” Dr. Larry Keefer notes, “It was always inspirational to see the organ scans of patients whose tumors were regressing—indeed disappearing—after dosage with the combination drug therapy Bob developed. Second-generation therapeutic agents he ingeniously designed show promise for even greater clinical utility.”

In longstanding collaborations with Dr. Anthony E. Pegg of the Pennsylvania State University College of Medicine and Dr. Henry S. Friedman of the Duke University Medical Center, Dr. Moschel’s research led to significant improvements in chemotherapy for brain tumors. His collaborative studies with Dr. Eileen Dolan (University of Chicago) showed that pretreatment of several cell lines with *O*<sup>6</sup>-benzylguanine enhanced the cytotoxicity of the chemotherapeutic agent cisplatin. This pretreatment approach might improve the prognosis of patients with head and neck, ovarian, testicular, or lung cancer who are routinely treated with cisplatin.

Dr. Moschel’s team also used site-specific mutagenesis techniques to study the chemical and biological effects of carcinogen damage to DNA and the role played by DNA repair mechanisms in mediating these effects. In a collaboration with Dr. Lisa Peterson (University of Minnesota), they demonstrated that a pyridyloxobutylguanine adduct, which plays an important role in tobacco-induced lung carcinogenesis, is highly mutagenic in both *Escherichia coli* and human cells. Subsequent studies suggested that differences in the repair of this adduct by mammalian proteins may translate into differences in sensitivity that these cells exhibit to tobacco-specific nitrosamines.

In addition to conducting research, Dr. Moschel was a member of both the Chemistry and Structural Biology Faculty and the Molecular Targets Faculty. During his career, he published more than 100 scientific papers in prestigious journals, such as *Cancer Research*, *Proceedings of the National Academy of Sciences USA*, *Cancer Chemotherapy and Pharmacology*, *Carcinogenesis*, *Chemical Research in Toxicology*, and *Journal of Organic Chemistry*. In addition, Dr. Moschel also served on the editorial advisory board of *Chemical Research in Toxicology* and was a member of the American Cancer Society’s Peer Review Committee on Carcinogenesis, Nutrition, and the Environment.

Dr. Moschel will be remembered by his friends and colleagues as a kind and gentle man whose research has led to new treatments for brain tumor patients and renewed hope for their families.

## Technology Transfer and the CCR Investigator

Technology transfer became a federal mission with passage of the Stevenson-Wydler Technology Innovation Act in 1980, which required federal laboratories to devote 0.5% of their budgets to technology transfer and those with budgets of more than \$20 million to establish an office of research technology applications. In 1986, this law was expanded by the Federal Technology Transfer Act, which empowered industry collaboration with federal employees through the use of Cooperative Research and Development Agreements (CRADAs). These laws were further extended in 1995 with the National Technology Transfer and Advancement Act and in 2000 with the Technology Transfer Commercialization Act, which established incentives for investigators, expedited CRADA negotiations, and allowed licensing of preexisting inventions.

The NCI has been actively working with many corporate partners who have successfully moved our research into products for patient use or use in health care facilities. Over the years, thousands of licensing agreements have been executed on NCI technologies, which transfer NCI inventions to the private sector for further research and development and potential commercialization that can lead to public health benefits. Licenses are granted in exchange for royalty payments and licensing fees. Biomedical research at the NIH is licensed through the Office of Technology Transfer (OTT). A report by the Department of Commerce states that the NIH's royalties accounted for nearly 70% of the total invention royalties received by the federal government, and 11 of the top 20 commercially successful inventions at NIH were based on NCI technologies. These include the HIV drugs Videx, Hivid, and Prezista, the cancer treatment drugs Taxol and Fludara, and the immunosuppressive drug Zenapax.

### Understanding Patents

A patent gives the inventor an exclusive right to develop an invention for a number of years, usually 20, in exchange for publicly disclosing the invention. To qualify for a patent, an invention must demonstrate novelty, usefulness, and non-obviousness. Furthermore, an invention must be either a process, a machine, a manufacture, a composition of matter, or any new and useful improvement thereof. If an invention were publicly disclosed prior to filing a patent application, it would no longer be considered novel, and the public disclosure could prevent the invention from being patented. All foreign patent rights are also lost on the day the information is disclosed. Many times a company will choose not to license a product without foreign patent protection. Additionally, all rights within the United States will be lost if a patent application is not filed within 12 months of the disclosure. Common ways that researchers inadvertently forfeit their patent rights include presentations, abstracts, journal articles, methods detailed in grant applications, theses, e-mails, and even certain conversations.

Sometimes commercialization and technology transfer are best accomplished without patent protection. At times, technologies or "know-how" is most appropriately transferred to the private sector through publication. For some technologies, patenting and licensing are costly, unnecessary, and could hinder their dissemination and application. Surgical procedures are a good example of the sort of technology that doesn't need patent protection. The vast majority of CCR's new inventions may be classified as research tools, for example, mouse models, antibodies, and cell lines, best distributed broadly under biological materials licenses without patent protection. This strategy encourages the distribution of these technologies at nominal

costs to the research community. However other technologies that have significant time and cost associated with their development, such as those with preventive, diagnostic, or therapeutic uses, may require patent protection for commercial product development.

#### **The CCR Office of Policy and Intellectual Property**

The CCR Office of Policy and Intellectual Property provides advice and guidance to the Center's Directors, senior staff, and others members of the CCR scientific and administrative community on issues relating to collaborative agreements, intellectual property, ethics, policy, and regulatory, judicial, and legislative issues. The goal of the office is to facilitate basic, translational, and clinical collaborations with the pharmaceutical industry and develop collaborative relationships with other agencies and organizations both nationally and internationally. The Office is the Center's principal contact with the NCI Technology Transfer Center (TTC), NIH Office of Technology Transfer (OTT), NIH Office of General Counsel (OGC), and NCI Ethics Office. The Office works closely and partners with TTC and OTT on issues relating to the CCR intellectual property portfolio, reviews and approves all CCR patent filing decisions, promotes technology, supports key technology development initiatives, and facilitates industrial basic, clinical, and translational research collaborations. The Office is involved with outreach for the CCR by strategizing and promoting collaborations with industry, academia, and other government institutes and agencies, facilitating the technology transfer process and ensuring that regulatory and training requirements are met. The Office also plays a role in conflict investigation and resolution to ensure that CCR objectives are best met.

Investigators are encouraged to contact the Office if they need assistance or advice on how best to understand and navigate the seemingly complex technology development process at the NIH.

If you have an interesting invention, talk to your NCI technology transfer specialist early to see if a patent should be pursued. To avoid loss of patent rights, also notify the specialist before any talk or publication that would disclose the invention and obtain signed confidential disclosure agreements where appropriate. Note that proper record keeping is essential for the ability to obtain a patent. Preferably, experiments should be recorded in bound notebooks with consecutively numbered pages. The entries should be signed and dated by the researcher each day and periodically witnessed by at least one person who is not an inventor with a notation such as "disclosed to and understood by me this \_\_\_\_ day of \_\_\_\_\_, 2007." With this type of record keeping, a patent application can seldom be challenged on the date an invention was developed.

The successful patenting and licensing of inventions not only contribute to the NCI mission by encouraging further development of promising new therapies, but also show the productivity and dedication of the CCR investigator to bring new therapies from the bench to the bedside. Patenting an invention

begins with filing an Employee Invention Report (EIR), which is reviewed by the NCI Technology Transfer Center (TTC) technology transfer specialist, the NIH OTT, and the NCI Technology Review Group (TRG) for patentability, marketing potential, and licensing interest. The combined recommendations are forwarded to the Center's Director for final filing decisions. After obtaining a filing date from the designated patent office, public disclosure of the invention can take place without jeopardizing its patentability.

The NCI receives royalties generated by licensing agreements on these government-owned patents and by biological material licenses. These funds can then be used to further stimulate promising research initiatives. Through licensure of inventions, individual investigators can directly receive a percentage of the royalties and may also be eligible for Federal Technology Transfer Awards. In 2006, the CCR filed 121 new EIRs, received 37 new patents, and

executed 81 new licenses, representing 33%, 40%, and 32%, respectively, of the totals for the NIH.

For more information on patents or procedures please visit the following Web sites:

The NCI Technology Transfer Branch: <http://ttc.nci.nih.gov>

NIH Office of Technology Transfer: <http://www.ott.nih.gov>

The US Patent and Trademark office: <http://www.USPTO.gov>.

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## ■ CANCER AND CELL BIOLOGY

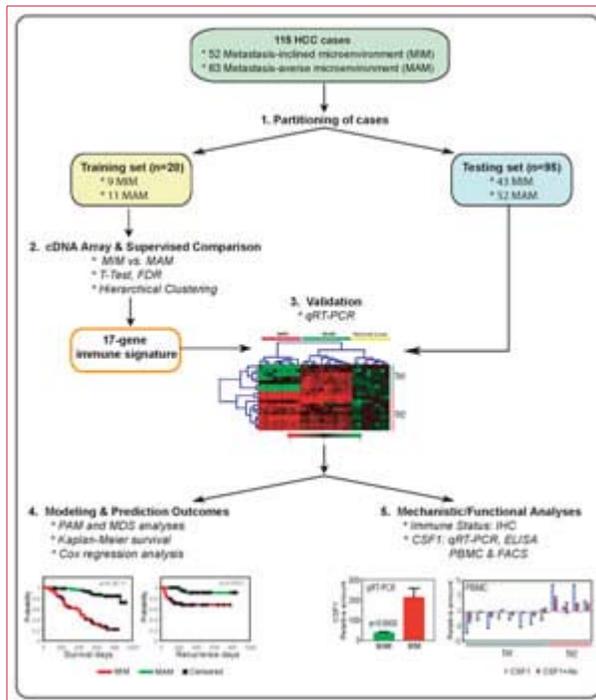
### **A Unique Immune-related Metastasis Signature of the Hepatic Microenvironment**

Budhu A, Forgues M, Ye QH, Jia HL, He P, Zanetti KA, Kammula US, Chen Y, Qin LX, Tang ZY, and Wang XW. Prediction of venous metastases, recurrence, and prognosis in hepatocellular carcinoma based on a unique immune response signature of the liver microenvironment. *Cancer Cell* 10: 99–111, 2006.

**H**epatocellular carcinoma (HCC) is typically associated with an extremely poor prognosis. The reason for this is the highly vascular nature of HCC tumors, which increases their propensity to spread and invade neighboring or distant tissues. Intra-hepatic metastases, especially venous metastases, are a major hallmark of HCC.

Recently, we developed a gene expression signature specific to primary HCC tumor specimens that predicted prognosis and venous metastases with 78% overall accuracy (Ye QH et al. *Nat Med* 9: 416–23, 2003). Since HCC is usually present in inflamed fibrotic and/or cirrhotic liver with extensive lymphocyte infiltration due to chronic hepatitis, it is possible that HCC metastatic propensity may be determined and/or influenced by the local tissue microenvironment of the host.

To determine the role of the hepatic microenvironment in HCC metastasis, we compared the gene expression profiles of noncancerous hepatic tissue samples obtained from areas surrounding tumors in (1) patients with primary HCC accompanied by venous metastases or confirmed extra-hepatic metastases by follow-up, which we termed metastasis-inclined microenvironment (MIM) samples and (2) patients with HCC without detectable metastases, which we termed metastasis-averse microenvironment (MAM) samples (**Figure 1**). We first conducted gene expression profiling studies of a subset of MIM and MAM samples from this cohort using cDNA microarray.



**Figure 1.** Schematic of the search for a metastasis-associated signature in the hepatic microenvironment. Twenty noncancerous hepatic tissue samples from areas surrounding tumors, characterized as MIM (metastasis-inclined microenvironment) or MAM (metastasis-averse microenvironment) samples, were analyzed by cDNA microarray (Step 1). A metastasis signature composed of 17 immune-related genes, associated with T helper cell type 1 (Th1)- and Th2-like cytokines, was significantly and differentially expressed in samples with metastasis (Step 2). Following validation by quantitative real-time polymerase chain reaction (qRT-PCR) (Step 3), prediction analysis of microarrays (PAM), multi-dimensional scaling (MDS), Kaplan-Meier survival analysis, and Cox proportional hazards modeling demonstrated accurate classification of patients with metastasis and prediction of outcome based on the 17-gene signature (Step 4). The pro-inflammatory status of metastasis samples was confirmed by immunohistochemistry (IHC), and a significant increase in the abundance of macrophage colony stimulating factor type 1 (CSF1) in metastasis samples was shown by qRT-PCR and ELISA (Step 5). Peripheral blood mononuclear cells (PBMC), incubated with recombinant CSF1, recapitulated the significant Th1-Th2 cytokine shift observed in metastasis samples, indicating that CSF1 may play a role in promoting the metastatic phenotype (Step 5). In addition, fluorescence-activated cell sorting (FACS) suggested that CSF1 induced these cytokine shifts in T-cell populations (Step 5). Ab, antibody; T-Test, student's t test; FDR, false discovery rate.

We identified a unique change in the gene expression profiles associated with a metastatic phenotype. Furthermore, using the same subset of MIM and MAM samples used in the microarray, we constructed a refined expression signature containing 17 genes (*IL1A*, *IL1B*, *IL2*, *IL12A*, *IL12B*, *IFNG*, *TNFA*, *IL15*, *IL4*, *IL5*, *IL8*, *IL10*, *HLA-DR*, *HLA-DPA*, *ANXA1*, *PRG1*, and *CSF1*), which we determined by quantitative real-time polymerase-chain-reaction analyses (qRT-PCR). This signature was validated by an independent cohort of 95 MIM and MAM samples and could successfully predict both venous metastases and extra-hepatic metastases by follow-up with greater than 92% overall accuracy. Moreover, the prognostic performance of this liver microenvironment signature was superior to and independent of other available clinical parameters for determining patient survival or cancer recurrence. The lead signature genes were associated with the cellular immune and inflammatory responses. Consistently, predominant changes in T helper cell type 2 (Th2)-like cytokine responses, favoring a humoral anti-inflammatory/immunosuppressive microenvironmental condition,

occur in MIM samples. Macrophage colony stimulating factor type 1 (CSF1) may be one of the cytokines overexpressed in the liver milieu that is responsible for this shift.

These findings suggest that the inflammatory status of the hepatic milieu, whether influenced by viral-hepatitis-mediated liver damage or individual genetic constitution, in addition to the metastatic potential of the tumor cells, plays an important role in promoting HCC tumor progression and venous metastases. In addition, this signature may be clinically useful for identifying HCC patients who may benefit from certain post-surgical treatments to prevent metastases and/or recurrence.

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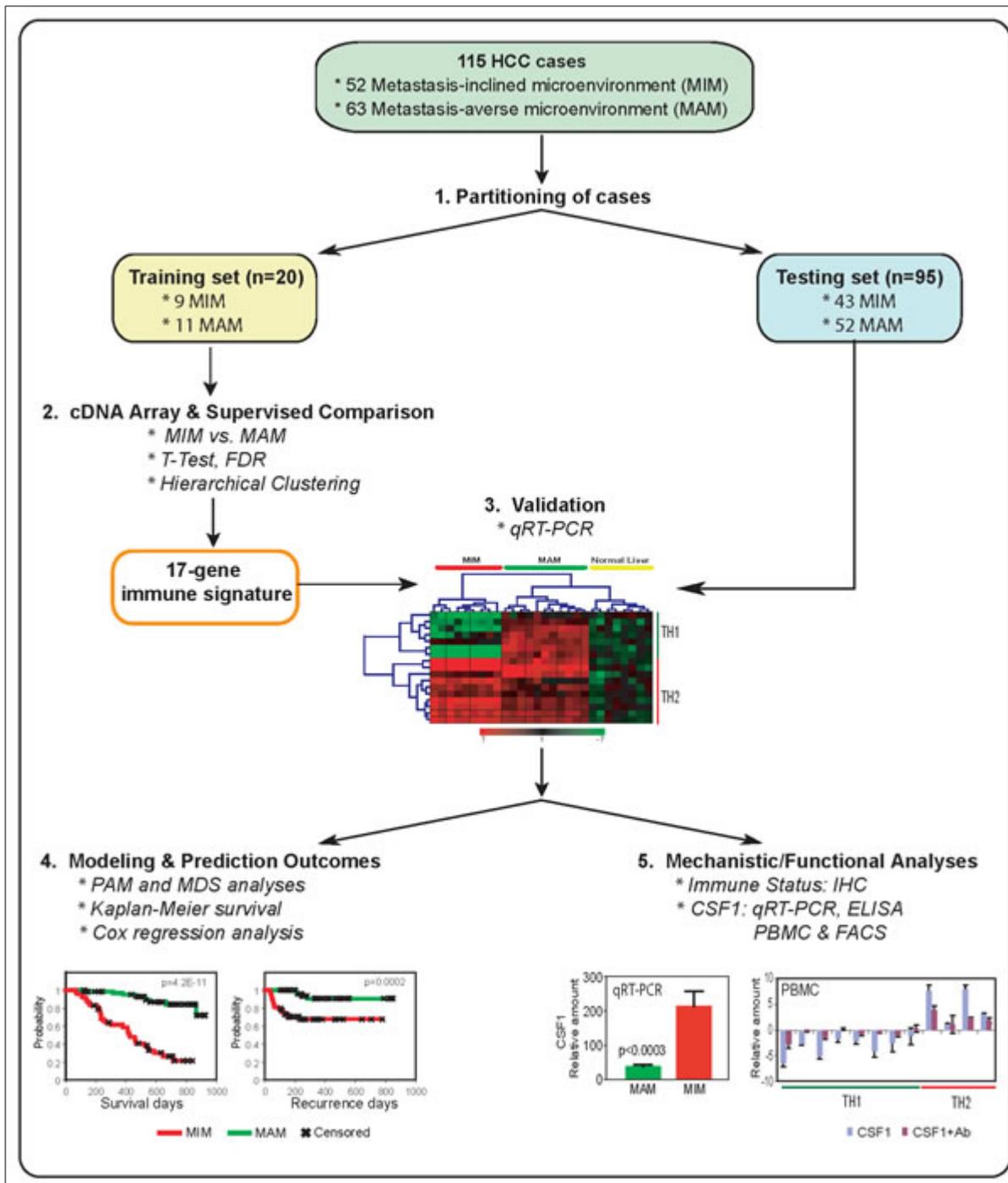
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## ■ CARCINOGENESIS

### Tumor Cells and Stroma CLIC to Promote Cancer Progression

Suh KS, Crutchley JM, Koochek A, Ryscavage A, Bhat K, Tanaka T, Oshima A, Fitzgerald P, and Yuspa SH. Reciprocal modifications of CLIC4 in tumor epithelium and stroma mark malignant progression of multiple human cancers. *Clin Cancer Res* 13: 121–31, 2007.

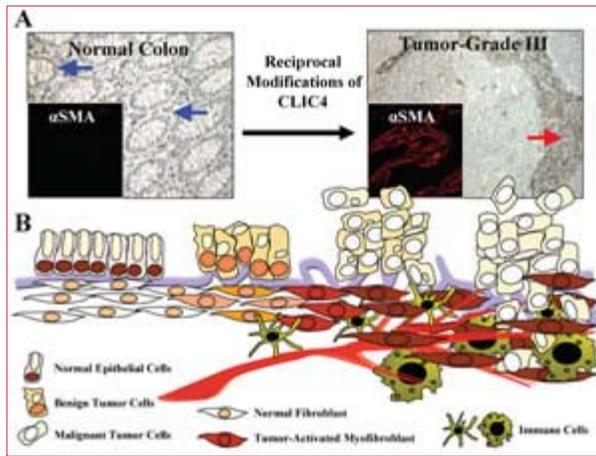
Intracellular chloride controls organelle volume, pH, and electrogenic balance and is crucial to regulate the integrity of intracellular organelles. Previous reports suggest that the regulation of chloride transport can influence tumor development and progression, but the changes may be specific to particular tumors and chloride channel families. We now report chloride intracellular channel-4 (CLIC4) has a more generalized pattern of cancer-associated changes in multiple human cancers.

CLIC4, one of seven members of the CLIC family, was discovered in a search for p53-regulated genes in differentiating keratinocytes (Fernandez-Salas E et al. *J Biol Chem* 274: 36488–97, 1999). Uniquely, soluble forms of CLIC proteins in the cytoplasm function in cell signaling pathways but undergo molecular and structural modifications in response to specific stimuli to autoinsert into the cellular/organelle membrane, where they behave as an anion channel or channel regulator. Therefore, CLIC proteins appear to be multifunctional, having both soluble and membrane activities. Cytoplasmic CLIC4 translocates to the nucleus in cells undergoing growth arrest or apoptosis in response to multiple stimuli, including metabolic or cytotoxic stress or physiological growth inhibitors. A C-terminus functional nuclear localization signal regulates nuclear trafficking. Overexpression of CLIC4 in the nucleus causes cell cycle arrest and accelerates apoptosis. CLIC4 is a direct downstream target of both p53 and c-Myc, two mediators of cancer pathogenesis in multiple tumors (Shiio Y et al. *J Biol Chem* 281: 2750–56, 2006) and is required for blood vessel tubular morphogenesis (Bohman S et al. *J Biol Chem* 280: 42397–404, 2005). These discoveries prompted us to evaluate the changes in *CLIC4* integrity, transcript and protein expression,

and the subcellular localization of its product in a series of human tumors and test the impact of the *in vivo* results on tumor growth in experimental models.

In analyses of cDNA and tumor lysate arrays of matched human normal and tumor tissues representing all major human solid tumors, CLIC4 expression was often reduced in the tumor extracts, particularly in ovary, renal, and breast cancers, but a specific expression pattern did not emerge. A more consistent pattern of change was detected in immunostained tissue arrays from multiple human solid epithelial cancers. Although CLIC4 was abundant and largely located within the nucleus in normal epithelium, CLIC4 was excluded from the nucleus and markedly reduced in the tumor epithelium. Conversely, CLIC4 was not highly expressed in the stroma of normal tissues but markedly upregulated in the tumor stroma, having been associated with myofibroblast conversion as indicated by co-expression of alpha smooth muscle actin ( $\alpha$ SMA). Thus, reciprocal modifications of CLIC4 in distinct tumor compartments would be difficult to detect in materials derived from whole tumor lysates. Transcript sequences of *CLIC4* from the human EST database and manual sequencing of cDNAs from NCI60 human cancer cell lines failed to reveal deletions or mutations in the gene, suggesting other genetic (e.g., methylation), post-transcriptional (e.g., mRNA stability), or post-translational (e.g., phosphorylation) changes may be responsible for the altered molecular expression of CLIC4 in cancer epithelium.

In several tumor types studied, the extent to which CLIC4 was lost in tumor epithelium and upregulated in tumor stroma directly correlated with the stage of tumor progression (Figure 1). To test the functional relevance of CLIC4 changes in tumors, we injected human breast cancer cells into nude mice as subcutaneous xenografts. Inducing CLIC4 overexpression in tumor cells and, particularly, the nuclei of tumor cells by adenovirus transduction inhibited tumor growth. In contrast, grafting breast cancer cells together with fibroblasts engineered to overexpress CLIC4 enhanced tumor growth. These engineered fibroblasts upregulated  $\alpha$ SMA in response to CLIC4 overexpression *in vitro*, and the xenografts were rich in myofibroblasts *in vivo*. When human breast cancer cells were co-cultured with fibroblasts, CLIC4 was not detected in cancer cell foci but was upregulated in fibroblasts surrounding cancer foci along with  $\alpha$ SMA. Temporally, it appeared that CLIC4 was upregulated prior to the appearance of  $\alpha$ SMA in fibroblasts surrounding tumor foci. Thus, CLIC4 participates in the crosstalk between tumor cells and their surrounding stroma to induce a microenvironment conducive to enhanced growth, and a compartment-directed CLIC4 expression profile, in conjunction with the  $\alpha$ SMA profile, may be a useful addition to the diagnostic criteria in marking/grading tumors. Further, CLIC4 may be a novel molecular target with significant therapeutic potential for the following reasons: (1) CLIC4 reduction in the tumor mass is a consequence of epigenetic factors and therefore may be reversible, which could impede tumor growth. (2) CLIC4 is specifically excluded from the nucleus of cancer cells and not normal cells, so restoring expression and nuclear localization may be selectively toxic to tumor cells. (3) Modification of stromal CLIC4 expression may alter myofibroblast activity and/or angiogenesis in the tumor microenvironment and serve to diminish host factors that are recruited by tumor cells to enhance their growth.



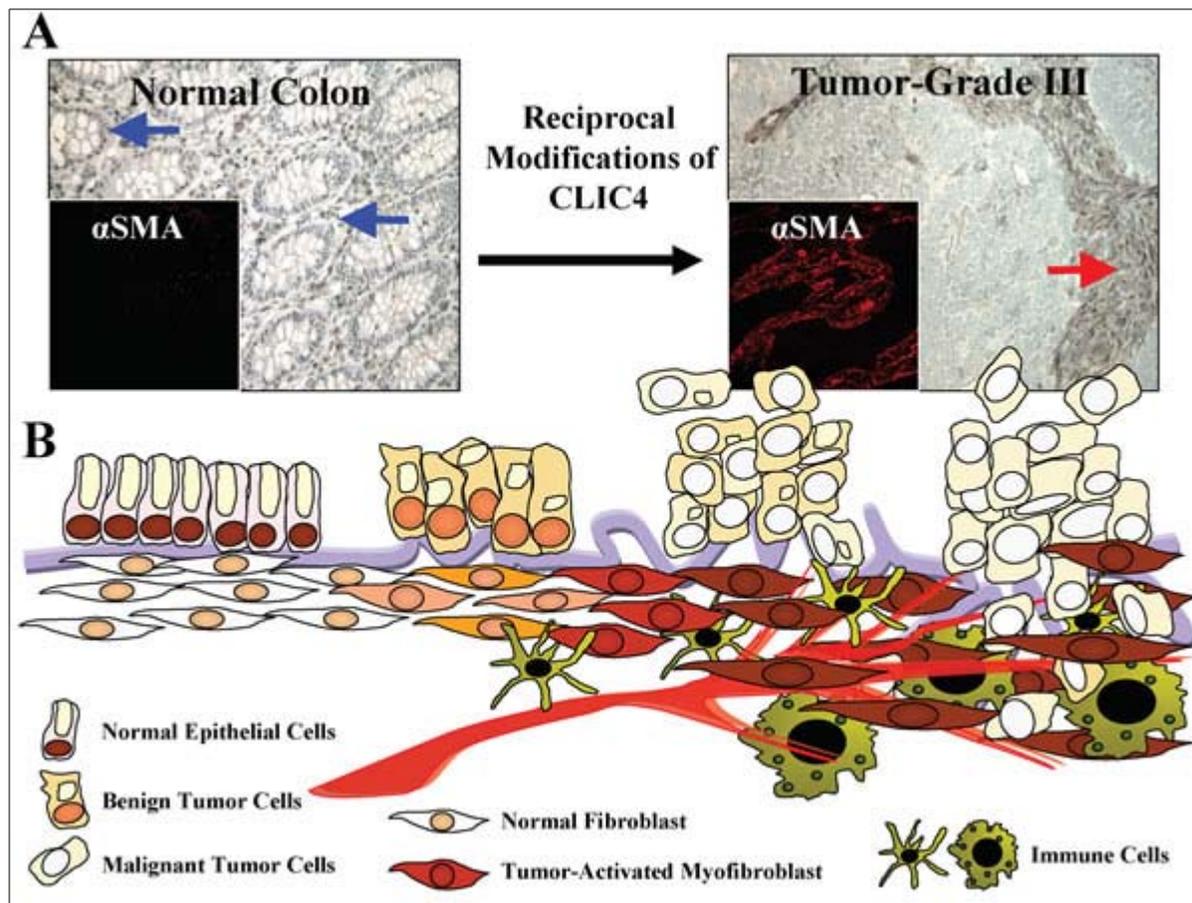
**Figure 1.** Reciprocal modifications of chloride intracellular channel-4 (CLIC4) in tumor epithelium and stroma directly correlate with tumor progression. *A)* Immunostaining of CLIC4 in tissue sections from normal colon showing the predominant localization of CLIC4 in nuclei of crypt cells and lamina propria (blue arrows). In contrast, CLIC4 is excluded from tumor epithelium in advanced colon cancer and upregulated in tumor stroma (red arrow) where it is co-expressed with alpha smooth muscle actin ( $\alpha$ SMA). *B)* Diagram depicting the step-wise reciprocal modifications of CLIC4 (represented by brown color) in epithelial cells and stromal fibroblasts over the course of the multistage development of cancer at multiple organ sites.

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■ CHEMISTRY

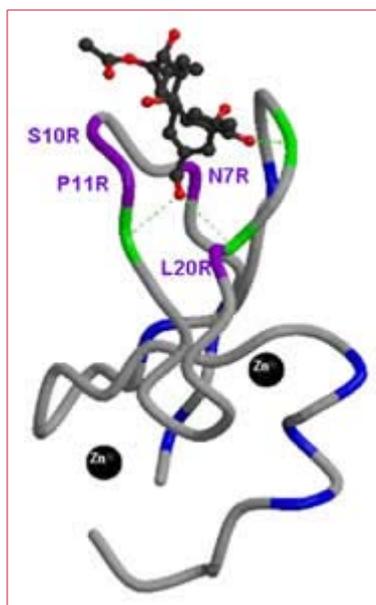
**Understanding Why the “Atypical” Protein Kinase C Isoforms Do Not Bind Phorbol Esters**

Pu Y, Peach ML, Garfield SH, Wincovitch S, Marquez VE, and Blumberg PM. Effects on ligand interaction and membrane translocation of the positively charged arginine residues situated along the C1 domain binding cleft in the atypical protein kinase C isoforms. *J Biol Chem* 281: 33773–88, 2006.

The phorbol esters, classic tumor promoters, function as ultrapotent analogs of sn-1,2-diacylglycerol (DAG), the ubiquitous second messenger generated through the breakdown of phosphatidylinositol 4,5-bisphosphate. The recognition motif for the phorbol esters and DAG is the C1 domain, a 50 amino acid long zinc finger structure. Protein kinase C (PKC) constitutes the best known class of signaling proteins containing C1 domains, which represent hydrophobic switches. Phorbol ester/DAG inserts into a hydrophilic cleft in an otherwise hydrophobic surface on the C1 domain, providing a hydrophobic cap for this cleft and favoring insertion of the hydrophobic face of the C1 domain into the lipid bilayer. This insertion drives both the conformational change of PKC, causing its activation, as well as its membrane translocation, controlling its access to substrates.

PKC is a compelling therapeutic target both for cancer and a range of other conditions. Within the CCR, the Laboratories of Cancer Biology and Genetics and of Medicinal Chemistry bring together biological and chemical methodologies to understand ligand interactions with C1 domains and to exploit this understanding to develop therapeutic leads. This effort has led to the design of DAG lactones that have affinities approaching those of phorbol esters and that provide powerful chemical tools for probing biological questions. In contrast to the classical and novel PKCs, the “atypical” PKC isoforms zeta and iota have C1 domains that are phorbol ester/DAG unresponsive. We have now begun to apply the lessons from the C1 domains of the classical and novel PKC isoforms to understand the nature of these “unresponsive” C1 domains.

The C1 domains of PKC zeta and iota are distinguished by a high net positive charge, arising from four arginines rimming the binding cleft. To probe their potential role, we started with the phorbol ester-binding C1b domain of PKC delta ([Figure 1](#)) and mutated the corresponding residues to arginine singly or in combination. Individually, mutation caused only a modest loss of binding affinity, a modest decrease in membrane translocation of the C1 domain in response to phorbol ester, and an enhanced requirement for anionic membrane phospholipids. Upon multiple mutations, binding and translocation were progressively abolished, yielding a C1 domain that behaved similarly to those of PKC zeta and iota.



**Figure 1.** The structure of phorbol ester bound to the C1b domain of protein kinase C (PKC) delta is shown. The phorbol ester is portrayed as a ball-and-stick representation, with the carbon atoms in black and the oxygen atoms in red. The backbone of the zinc finger is indicated in grey. The four residues N7, S10, P11, and L20, which are present as arginines in PKC zeta and iota, are purple. The residues that hydrogen bond with the phorbol ester are in green (the hydrogen bonds are shown as green dotted lines), and the other positively charged residues are blue.

To determine whether other residues in the C1 domains of PKC zeta and iota, independent of these arginines, could abrogate phorbol ester responsiveness, we reciprocally mutated the four arginines in the C1 domains of PKC zeta and iota to the corresponding residues found in the C1b domain of PKC delta. The mutated C1 domains gained phorbol ester responsiveness, undergoing translocation in response to phorbol ester, albeit with potencies approximately 30-fold weaker than that of the C1b domain.

Computer modeling provides insight into the mechanism by which the arginine residues influence phorbol ester binding. The modeling predicts that the conformation of the binding cleft in the C1 domains of PKC zeta and iota is similar to that of the C1 domains that bind phorbol ester. However, the arginine residues along the rim of the binding cleft can swing into and occlude the binding pocket, thereby competing with the ligand for occupancy.

Approximately half of the 60 mammalian proteins with C1 domains have been described as phorbol ester unresponsive. Our results establish that the so-called phorbol ester unresponsive C1 domains fall into two categories, those retaining the intrinsic binding geometry of the cleft (e.g., PKC zeta and iota), and those that no longer contain such a binding site (e.g., Raf). An implication, supported by preliminary results, is that it may be possible to design ligands tailored to C1 domains such as that of PKC zeta and iota.

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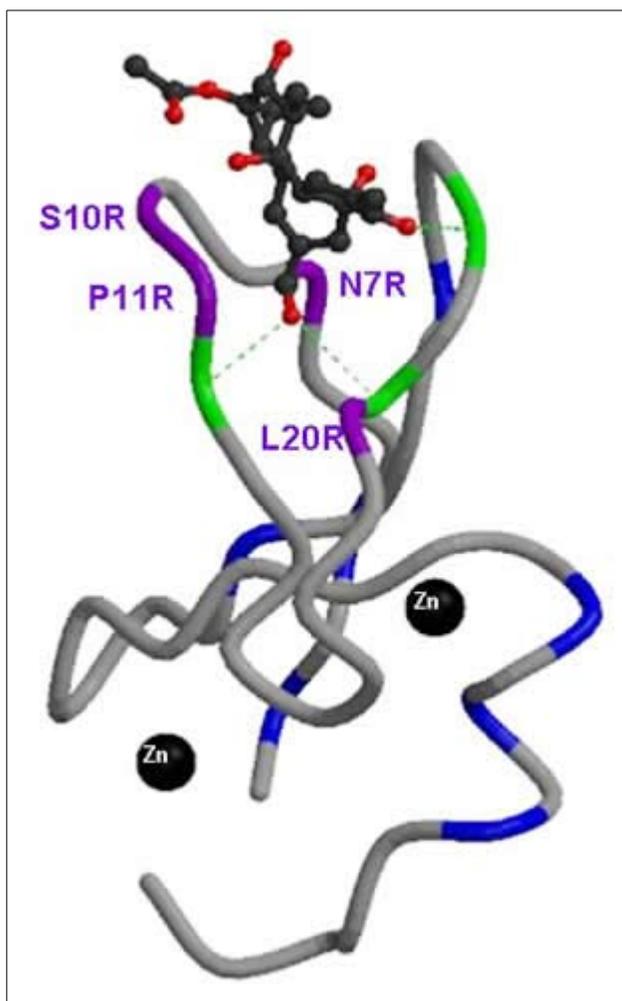
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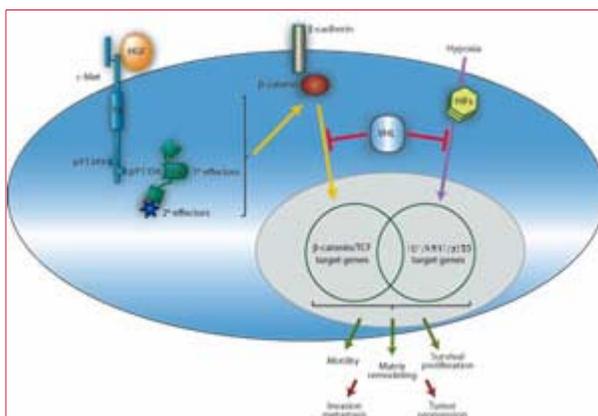
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■ MOLECULAR BIOLOGY

## Beta Catenin: A New Kidney Cancer Oncogene

Peruzzi B, Athauda G, and Bottaro DP. The von Hippel-Lindau tumor suppressor gene product represses oncogenic beta-catenin signaling in renal carcinoma cells. *Proc Natl Acad Sci U S A* 103: 14531–6, 2006.

Loss of von Hippel-Lindau (*VHL*) tumor suppressor gene function occurs in familial and most sporadic renal cell carcinoma (RCC) cases, resulting in the aberrant expression of genes that control cell proliferation, invasion, and angiogenesis. The molecular mechanisms by which *VHL* loss leads to tumorigenesis are not yet fully defined. The *VHL* gene product, pVHL, is part of an E3 ubiquitin ligase complex that targets hypoxia inducible factors for polyubiquitination and proteosomal degradation, implicating hypoxia response genes in RCC oncogenesis. *VHL* loss also allows robust RCC cell invasiveness and morphogenesis in response to hepatocyte growth factor (HGF), an important regulator of kidney development and renal homeostasis. Our recent analysis of the mechanism by which pVHL represses HGF-driven invasiveness has revealed another oncogenically relevant pVHL target:  $\beta$ -catenin (Figure 1).



**Figure 1.** Activation of the hepatocyte growth factor (HGF)/c-Met signaling pathway results in  $\beta$ -catenin tyrosyl phosphorylation, through interactions with the c-Met tyrosine kinase (TK) or with primary and/or secondary effectors. This results in the dissociation of  $\beta$ -catenin from E-cadherin in adherens junctions and its accumulation in the cytosol. If not rapidly ubiquitinated and degraded, cytosolic  $\beta$ -catenin translocates to the nucleus, where it regulates the transcription of genes that mediate processes that are known to contribute to tumorigenesis, malignancy, and metastasis. The product of the von Hippel-Lindau (*VHL*) tumor suppressor gene has been known to suppress RCC tumorigenesis through the ubiquitination and subsequent proteosomal destruction of hypoxia inducible factors (HIFs). As we recently reported, VHL protein is also critical for targeting cytoplasmic  $\beta$ -catenin for proteosomal destruction in renal epithelial cells; *VHL* gene loss in RCC thus promotes oncogenic signaling through both  $\beta$ -catenin and HIF pathways. ARNT, aryl hydrocarbon receptor nuclear translocator; TCF, T-cell factor; SH2, Src homology 2 domain.

HGF signaling between mesenchymal and adjacent epithelial cell compartments is a major driving force in embryonic kidney morphogenesis and differentiation, and inappropriate HGF pathway activation in cancer can resemble these epithelial-to-mesenchymal transitions (Birchmeier C et al. *Nat Rev Mol Cell Biol* 4: 915–25, 2003). Expression of HGF and its

receptor, c-Met, persists in the adult kidney, but striking changes occur in the quality of the response of renal epithelial cells to HGF stimulation upon completion of development. Morphogenic and proliferative responses are minimized, but HGF continues to protect kidney tissue from toxicity and stress, and it counteracts renal fibrosis. Many intracellular c-Met signaling pathways persist through development into adulthood, but how some signals are silenced to provide a homeostatic, as opposed to developmental or pathological, response remains unclear.

Under hypoxic conditions or when the *VHL* gene is mutated or lost, the hypoxia inducible factors that pVHL targets for degradation accumulate, leading to increased expression of hypoxia response genes that shift energy metabolism toward glycolysis and initiate angiogenesis through the increased production of proteins such as vascular endothelial growth factor, platelet-derived growth factor, and c-Met. Hypoxia also enhances HGF signaling through undefined mechanisms and, in turn, promotes invasive growth in cultured cells and mouse tumor models. Cultured *VHL*-negative RCC cells accumulate hypoxia inducible factors aberrantly and respond to HGF with increased motility, extracellular matrix invasion, and branching morphogenesis—responses typical of embryonic renal cells that are repressed in adulthood (Koochekpour S et al. *Mol Cell Biol* 19: 5902–12, 1999). These HGF-driven activities are abolished when wild-type *VHL* gene expression is reconstituted in RCC cells, directly linking loss of *VHL* function to an invasive tumor phenotype.

We recently elucidated the molecular mechanism by which pVHL represses HGF-driven RCC cell invasiveness, hypothesizing that pVHL negatively regulates  $\beta$ -catenin signaling downstream of c-Met in mature renal tubule epithelial cells and that *VHL* loss in RCC permits  $\beta$ -catenin to signal an aberrantly motile and invasive phenotype (Peruzzi B et al. *Proc Natl Acad Sci U S A* 103: 14531–6, 2006). Distinct roles for  $\beta$ -catenin have been established in the maintenance of intercellular adhesion and in the transcriptional activation of genes involved in normal growth and development; intracellular localization of  $\beta$ -catenin away from regions of cell-cell contact is correlated with the latter. Consistent with a shift in the balance of function from adhesion to signaling, we found that HGF stimulated the redistribution of  $\beta$ -catenin from peripheral to cytoplasmic and nuclear pools in *VHL*-negative RCC cells. In non-tumor, pVHL-positive, renal epithelial cells, *VHL* gene silencing was required to elicit a similar HGF-driven redistribution of  $\beta$ -catenin, matrix invasion, and cellular morphogenesis. Conversely, restoration of pVHL expression in RCC cells led to the repression of HGF-stimulated adherens junction disruption, cytoplasmic  $\beta$ -catenin stabilization, nuclear translocation, and target gene activation. Finally, ectopic expression of an ubiquitination-resistant  $\beta$ -catenin mutant bypassed wild-type *VHL* function, enabling HGF-driven invasion and morphogenesis in cells otherwise incapable of these responses. These findings identify  $\beta$ -catenin as a critical substrate of pVHL and as a novel target for biomarker and drug development in the effort to successfully treat metastatic RCC.

Oncogenic  $\beta$ -catenin signaling has been demonstrated in colon, breast, prostate, and lung carcinomas as well as melanoma (Polakis P. *Cell* 105: 563–6, 2001). In all of these cancers, failure to degrade cytoplasmic  $\beta$ -catenin protein is the common link to oncogenic signaling.

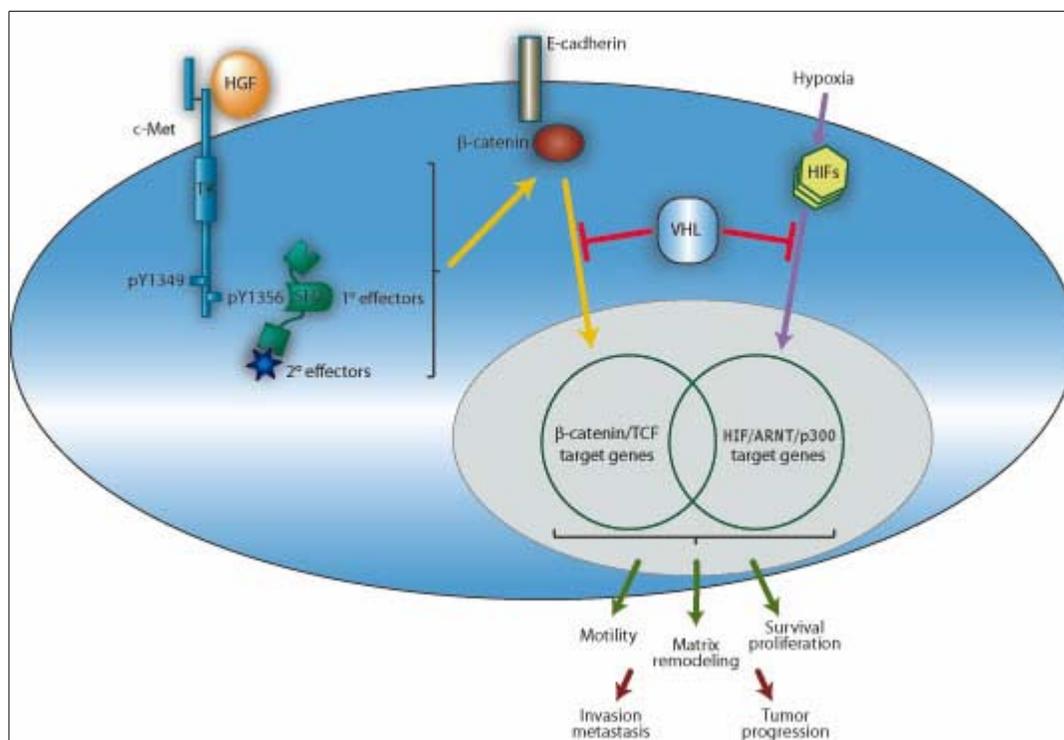
In colorectal cancer, mutations in genes involved in  $\beta$ -catenin ubiquitination occur in more than 90% of all tumors. In contrast, mutations in these genes are rare in non-colon cancers. Nonetheless, a wide variety of tumor samples show cytoplasmic and/or nuclear accumulation of  $\beta$ -catenin protein. RCC now joins the list of cancers in which  $\beta$ -catenin contributes to oncogenesis, but through an unexpected relationship with an E3 ligase component specifically lost in most renal cancers: pVHL.

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**Figure 1.** Activation of the hepatocyte growth factor (HGF)/c-Met signaling pathway results in  $\beta$ -catenin tyrosyl phosphorylation, through interactions with the c-Met tyrosine kinase (TK) or with primary and/or secondary effectors. This results in the dissociation of  $\beta$ -catenin from E-cadherin in adherens junctions and its accumulation in the cytosol. If not rapidly ubiquitinated and degraded, cytosolic  $\beta$ -catenin translocates to the nucleus, where it regulates the transcription of genes that mediate processes that are known to contribute to tumorigenesis, malignancy, and metastasis. The product of the von Hippel-Lindau (*VHL*) tumor suppressor gene has been known to suppress RCC tumorigenesis through the ubiquitination and subsequent proteosomal destruction of

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