Monday, 21 September 2009

Opening Session (Mon, 21 Sep, 08:55−10:55)

1 INVITED

Drug discovery in the p53 pathway

D.P. Lane. *Cancer Research UK University of Dundee, UK*

Small molecules that activate the transcriptional function of wild type p53 have proved active as anti-cancer drugs in preclinical models and are now entering clinical trial. The complexity of the upstream p53 signaling pathway offers many targets for the development of such activators and the action of Mdm2 inhibitors, kinase inhibitors, sirtuin inhibitors and low doses of actinomycin D will be described among other examples. The possibility of using combinations of molecules will also be analyzed as will the issues of toxicity and resistance expected from these types of molecules. One concern is that the activation of p53 might exert some specific harmful affect on normal tissues and another is that treatment with p53 activators will select for tumor cells in the population that have mutant p53.
What can be done in those cases where p53 is mutant? One approach is the search for molecules that re-activate mutant p53 proteins, though some have likened this to trying to unscramble eggs, recent discoveries in protein dynamic and protein folding pathways have been very exciting. Recent approaches to developing molecules that will chaperone mutant p53's to fold in the active state will be described with reference to recent crystallographic analysis.
A second conceptual approach to exploiting the frequent loss of p53 function in tumors is that of synthetic lethality. Might p53 mutant tumors be more susceptible to inhibitors of DNA repair as has been seen for BRCA1 mutant tumors for example? Finally the concept of using p53 activating molecules as chemo-protectives has been proposed. In this concept normal cells are arrested temporarily in GI by p53 activators such as the Mdm2 inhibitor Nutlin. These cells are thus protected from cytotoxic drugs active at G2/M such as taxol. Cells that lack p53 and are not therefore arrested by Nutlin would retain their sensitivity. In this model then the therapeutic index of such cytotoxic drugs for p53 mutant tumors would be enhanced by protection of normal tissues.

References

Klein C and Vassilev L T. B J Cancer 2004 91:1415.
Dey A, Verma CS and Lane DP. BJ Cancer 2008 98:4.

2 INVITED

New targeted treatments in oncology: the mammoths and the foxes

J.P. Armand[1]. [1]*Institut Claudius Regaud, Department of Medicine, Toulouse Cedex, France*

Taxotère was the last cytotoxic molecule of the XXth century closing forty years of research with a simple ambition to inhibit the cellular proliferation. Glivec first targeted molecule of the XXIth century constitutes the model of what it is advisable to call a targeted molecule nowadays. The aimed target is the real cause of the abnormality at the origin of the cancer and neither the effect which results from it. Two radically different technologies were developed targeting these various ways of signalisation: the inhibitors of tyrosine kinase (TKI) "the foxes", mostly small molecules, results of a complicated process of discovery, easy to make and to copy, very often administered by nonstop oral way, their size allow an access to intracellular targets. In this family we find Imatinib, Gefitinib, Erlotinib, Sugen 11258, Sorafenib. Their association in a classic contemporary chemotherapy is often sanctioned by a failure. Most of them are multitargetings (dirty) drugs. In contrast, more voluminous molecules "mammoths", antibodies monoclonaux which are of relatively fast discovery, particularly expensive to manufacture. They are difficult to reproduce. The real generics are then, difficult to introduce they are biosimilar. They are administered by intravenous way with immunogenic risks, they affect only membrane targets of surface and they develop their maximal activity in association with classic antimitotics. They are Rituximab, Trastuzumab, Cetuximab and Bevacizumab. Opposed to the foxes they are sometimes qualified as mammoths. The plan of Hanhan and Weinberg finds these two therapeutic useful tools in six fundamental mechanisms of the oncogénèse:
1. The independence towards the signals of proliferation
2. The metastatic dissemination
3. The loss of the cellular control.
4. The loss of the apoptotic way
5. The immortalisation of the cellular lineages.
6. The neo-angiogénèse.

In those 6 domains were developed at the same moment antibodies and inhibitors of tyrosine kinase either very specific as antibodies, or on the contrary multi-sites as TKI.
As any efficient drugs, those multi-targeted drugs have an efficiency which is dose dependent, but they have particulars toxicities dose dependent to as a specific target.
Their utilisation raise different questions: acquired and de novo resistance, dose adjustment, multitargeted drug, versus association of mono-targeted molecules, associations between mammoths and foxes.
Conclusion: the XXIth century has opened a therapeutic era radically different from the XXth century. To the scarcity of medicine cytotoxics opposes today a considerable number of molecules candidates in the course of preclinical evaluation. Clinical skills are critical at the early development period to select between those candidates.

Scientific Symposium (Mon, 21 Sep, 11:00−13:00)
The hypoxia pathway in tumour progression and therapy

3 INVITED

Oxygen sensing, HIF hydroxylases and new cancer targets

W. Kaelin Jr[1]. [1]*Dana Farber Cancer Center, Boston, USA*

Most successful drugs are small organic molecules that bind to, and inhibit, a specific protein within the cell (i.e. its target). Enzymes have historically proven to be attractive drug targets as they frequently have catalytic clefts or pockets that can bind to small organic molecules that possess drug-like properties. 2-oxoglutarate-dependent dioxygenases are a recently identified superfamily of oxygen-dependent enzymes that have now been linked to a number of biological processes, including cancer. For example, we and others showed that the alpha subunit of the heterodimeric transcription factor HIF (Hypoxia-inducible Factor) is hydroxylated by members of the PHD (also called EglN) family of prolyl hydroxylases, which are 2-oxoglutarate-dependent dioxygenases. Prolyl hydroxylation of HIFalpha leads to its recognition by the pVHL tumor suppressor protein, which earmarks HIF for proteasomal degradation. Under low oxygen conditions, or in cells lacking pVHL, HIF accumulates in its active form and transcriptionally activates ~200 genes that promote survival in a low oxygen environment, including genes that promote erythropoiesis. There are 3 PHD family members in humans although PHD2 (EglN1) appears to be the primary HIF prolyl hydroxylase under normal conditions and hypomorphic PHD2 mutations have been identified in families with familial polycythemia. Small molecule PHD inhibitors stimulate erythropoiesis in mice, monkeys, and man and have advanced to Phase 2 studies. We have identified HIF-independent roles for PHD1 and PHD3 in the control of cell proliferation and apoptosis, respectively. For example, PHD1 is induced by estrogen and appears to play an important role in the control of ER positive breast cancers as well as other cancers. Recently it was discovered that many histone demethylases are also 2-oxoglutarate-dependent dioxygenases. For example, we showed, with the help of Drs. Robert Klose and Yi Zhang, that RBP2 (JARID1A) is an H3K4 demethylase that belongs to this family. RBP2 binds to the RB tumor suppressor protein and some of RB's actions can be replicated in RB−/− tumor cells by inactivating RBP2. Genetic disruption of RBP2 inhibits tumor cell proliferation and promotes differentiation in vitro. Our preliminary data indicate that loss of RBP2 inhibits tumor formation in two genetically engineered mouse models.

4 INVITED

Mechanisms of tumour suppression by VHL

W. Krek[1]. [1]*Institute for Cell Biology, ETH Zurich, Zurich, Switzerland*

Mutations in the von Hippel-Lindau (VHL) tumor suppressor gene are causally linked to the development of various inherited and sporadically occurring tumors including clear cell renal cell carcinoma. Work over the last years has established key roles of the VHL gene product VHL in the regulation of diverse cellular processes (Frew et al., 2007). Their disruption is believed to endow susceptible cells with attributes to progress towards an aggressive tumorigenic potential. A commonly accepted tumor suppressor function of VHL relates to its role in hypoxia signaling, where it acts as a substrate recognition component of an ubiquitin ligase that targets hypoxia-inducible factor (HIF)α subunits for degradation. We have previously reported on a HIF-independent function of VHL, which is linked to its ability to bind to and stabilize microtubules (MTs) (Hergovich et al., 2003). The MT stabilization function of VHL is compromised by certain naturally-occurring VHL mutations and has been linked to primary cilium maintenance (Thoma et al., 2007) and suppression of renal cyst formation in mouse models (Frew et al., 2008), implying that regulation of MT stability by VHL is a critical aspect of its tumor suppressing activity. More recently, we have investigated whether other cellular processes that depend on

proper MT dynamics, in particular mitosis, are affected by loss of VHL function. We found that VHL localizes to the mitotic spindle and functions to suppress spindle mis-orientation and to promote chromosomal stability by positively regulating Mad2 mitotic checkpoint protein expression. An association between VHL inactivation, reduced Mad2 levels and increased aneuploidy was also found in human renal cancer, implying that this newly identified functions of VHL in promoting proper spindle orientation and chromosomal stability likely contribute to tumour suppression (Thoma et al., in press).

References

Frew, I.J., and Krek, W. (2007). Multitasking by pVHL in tumour suppression. Current Opinion in Cell Biology 19: 685–690.

Hergovich, A., Lisztwan, J., Barry, R., Ballschmieter, P., and Krek, W. (2003). Regulation of microtubule stability by the von Hippel-Lindau tumour suppressor protein pVHL. Nature Cell Biology 5: 64−70.

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Thoma, C.R., Toso, A., Gutbrodt, K.L., Schraml, P., Frew, I., Hergovich, A., Moch, H., Meraldi, P., Krek, W. (2009). VHL loss causes spindle mis-orientation and chromosome instability. Nature Cell Biology, in press.

5 INVITED

Hypoxia-inducible factor 1 in cancer pathogenesis and therapy

G.L. Semenza[1]. [1]*The Johns Hopkins University School of Medicine, Institute for Cell Engineering, Baltimore, USA*

Hypoxia-inducible factor 1 (HIF-1) regulates the transcription of many genes involved in key aspects of cancer biology, including immortalization, maintenance of stem cell pools, cellular dedifferentiation, genetic instability, vascularization, metabolic reprogramming, autocrine growth factor signaling, invasion, metastasis, and treatment failure. HIF-1 is a heterodimeric protein composed of a constitutively expressed HIF-1β subunit and an O2-regulated HIF-1α subunit. In animal models, forced HIF-1α overexpression is associated with increased tumor growth, vascularization, and metastasis, whereas HIF-1 loss-of-function has the opposite effect. Immunohistochemical detection of increased HIF-1α protein levels in tumor biopsy sections, or microarray detection of increased HIF-1 target gene expression, is a negative prognostic factor in many types of human cancer. These findings have validated HIF-1 as a therapeutic target.

A cell-based screening assay for drugs that inhibit HIF-1-mediated transcription revealed that cardiac glycosides, such as digoxin, inhibited: synthesis of HIF-1α protein; expression of HIF-1 target genes that regulate angiogenesis (VEGF), glucose transport (GLUT1), and glycolysis (HK1, HK2); and the growth of human hepatoma, lymphoma, and prostate cancer xenografts. Anthracycline compounds, such as doxorubicin, did not affect the levels of HIF-1α but instead inhibited the binding of HIF-1 to hypoxia-response element sequences in target genes such as VEGF and PDK1. Daily low-dose doxorubicin administration rapidly arrested the growth of prostate cancer xenografts and blocked the intratumoral expression of HIF-1-regulated angiogenic cytokines (VEGF, stem cell factor, and stromal-derived factor 1), tumor-induced mobilization of angiogenic cells into peripheral blood, and tumor vascularization.

Taken together, the large body of clinical and experimental data regarding the role of HIF-1 in human cancer pathogenesis suggests that the addition of HIF-1 inhibitors to therapeutic regimens may improve outcome in patients whose tumor biopsy reveals high levels of HIF-1α in a tumor type in which such overexpression is associated with increased mortality (bladder, brain, breast, cervical, colorectal, endometrial, gastric, gastrointestinal stromal cell, non-small cell lung, oropharyngeal squamous cell, ovarian, and pancreatic cancer). In particular, digoxin and other cardiac glycosides have been used to safely and chronically treat patients with congestive heart failure and epidemiological studies have associated such treatment with reduced incidence of bladder and kidney, breast, and prostate cancer.

6 INVITED

Hypoxia signalling, metabolism and cancer

J. Pouyssegur[1], J. Chiche[1], R. LeFloch[1], K. Ilc[1], D. Roux[1], C. Brahimi-Horn[1], N. Mazure[1]. [1]*Centre Antoine Lacassagne, CNRS-UMR, Nice Cedex 2, France*

Without nutrient sensing and feedback from the tissue microenvironment, fast growing cells of the developing embryo and of expanding tumors would rapidly outstrip the supply of nutrients and die. Although cells sense and respond to variations in the concentration of key nutrients, oxygen sensing has emerged, early on in evolution, as a central control mechanism of energy metabolism and vasculogenesis. At the heart of this regulatory system is the Hypoxia-Inducible Factor, HIF, which controls, among other gene products, the expression of VEGF-A and Angiopoietin-2, two key angiogenic factors. This finding has placed the hypoxia-signaling pathway at the forefront of nutritional control. HIF can induce a vast array of gene products controlling glycolysis, intracellular pH (pHi), angiogenesis, cell migration and invasion, and so has become recognized as a strong promoter of tumor growth. The pro-invasion feature of HIF, measured by stimulation of Epithelial-Mesenchyme-Transition, could be seen as an integrated program 'designed' for migration-induced nutrient-search, as in microorganisms. It is therefore not surprising that HIF also promotes access to another source of nutrients by inducing macro-autophagy. In the context of this symposium, we will highlight some of the HIF-induced gene products that participate in tumor adaptation, resistance and progression in a nutrient-depleted and acidic microenvironment.

First we will demonstrate that the two HIF-induced 'BH3-only'-proteins (BNIP3, BNIP3L/NIX), in contrast to the current believe, do not trigger cell death but, by inducing macro-autophagy, stimulate tumor cell survival. We propose a model in which the low-affinity BH3-domains of hypoxia-induced BNIP3/BNIP3L have been 'designed' to induce autophagy. They can disrupt the Beclin1-Bcl2 and Beclin1-Bcl-XL complexes without inducing cell death.

Second, we will show how tumor cells by expressing two HIF-dependent membrane-bound carbonic anhydrases, CAIX and CAXII, acidify the extracellular milieu, and ensure a more alkaline intracellular pH favoring migration, survival and growth in a hostile acidic microenvironment. Inducible knock down of CAIX and CAXII by short hairpin interfering RNA is able to severely reduce growth of colon adeno-carcinoma spheroids and tumors in *nude* mice.

Third, HIF-induced glycolysis in most hypoxic tumor cells is essential to ensure maintenance of ATP levels for growth and cell survival. Two MonoCarboxylate Transporters MCT-1 and MCT-4, stabilized in the plasma membrane by the common chaperon basigin/CD147, play a key role in cancer metabolism. These transporters are critical for lactic acid export. Inactivation of MCT-1 the only form expressed in Ras-transformed fibroblasts abolishes tumor growth. We will show that the hypoxia-induced MCT-4 is critical for tumor resistance, survival and tumor growth in human epithelial tumors. Combined inactivation of both MCT-1 and MCT-4 is required to collapse tumor growth.

We propose that appropriate exploitation of these HIF-regulated proteins and new validated cancer targets, which control exacerbated tumor metabolism and intracellular pH, will be at the forefront of anti-cancer therapy.

Scientific Symposium (Mon, 21 Sep, 11:00−13:00)
Symptom clusters in cancer therapy

7 INVITED

What's new, what's best in symptom management?

C. Miaskowski[1]. [1]*University of California San Francisco, Department of Physiological Nursing – School of Nursing, San Francisco, USA*

The majority of symptom management research in oncology has focused on an evaluation of single symptoms (e.g., fatigue, pain, nausea, vomiting). However, clinical experience suggests that oncology patients rarely present with a single symptom. Therefore, the new frontier in symptom management research is the evaluation of symptom clusters. This presentation will provide an overview of the field of symptom cluster research. The evolving definition of a symptom cluster will be discussed and methodologic approaches to symptom cluster research will be described. In addition, this presentation will summarize recent findings from symptom cluster research that can be used in clinical practice. Current research findings in symptom cluster research suggest that the development of symptom clusters depends on the patient's cancer diagnosis as well as the type of cancer treatment the patient receives. In addition, recent evidenced suggests that some symptoms within a specific symptom cluster are relatively stable across a course of cancer treatment. The presentation will conclude with a discussion of future directions for research on symptom clusters.