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Editorial: October 2005

We are pleased to announce in this issue that prof. René Bernards was the winner of the 2005 Pezcoller Foundation-FECS Recognition for Contribution to Oncology.

René Bernards is head of the Molecular Carcinogenesis Department of the Netherlands Cancer Institute in Amsterdam.

The Selection Committee met in Bruxelles in April 2005 and included the following: Harry Bartelink as chairperson, Luigi Chieco Bianchi, Juan Foubert, William J. Gullik, Harald Zur Hausen, voting members and Kathleen Vandendael, Alberto Costa and Gios Bernardi as non-voting members.

The scientific committee proposed Dr. René Bernards as the winner of this year's Pezcoller FECS Award, on the one hand for his pivotal work in developing a technique to predict treatment outcome in breast cancer patients and on the other hand, probably even more important, for his work on a system which allows us to develop new drugs for cancer patients in the short term, which can be tested rapidly at the clinic.

Professor Bernards is using innovative techniques in his research to study fundamental cell processes. In the last four years, his group has focused on the development of new tools to carry out genome-wide genetic screens for the identification of genes that act in cancer-relevant pathways.

His laboratory employs both retroviral cDNA expression libraries in gain-of-function genetic screens as well as vectors that mediate stable suppression of gene expression through RNA interference to carry out large-scale loss-of-function genetic screens.

He was also responsible for the implementation of DNA microarray technology to study gene expression patterns in breast cancer.

17th Simposium Pezcoller
Trento, 16-18 June 2005

This work has demonstrated that breast cancer patients with poor prognosis have a distinctive "poor prognosis" gene expression signature.
This gene expression signature has stronger predictive power then any of the currently used diagnostic tools for predicting disease outcome in breast cancer and is currently being used to determine the need for adjuvant chemotherapy for patients with breast cancer.
René Bernards will hold an important lecture on his work at the ECCO 13 Conference in Paris on October Monday 31, entitled 'Functional genetic approaches to cancer'.

The International Selection Committee for the 2006 Pezcoller Foundation-AACR International Award for Cancer Research will be held in Philadelphia in December 2005.

We are also announcing the next 18th Pezcoller Symposium to be held in Trento (Italy) 27-29 June, 2006 entitled 'Tumor Microenvironment: heterotypic interactions' with Robert Weinberg and Enrico Mihich as Co-Chairmen.

But the most important part of this issue is the paper by Lewis C. Cantley, the winner of the last Pezcoller Foundation-AACR International Award for Cancer Research entitled 'The role of Phosphoinositide 3-Kinase in Cancers'.
We are very grateful to Lewis Cantley who gave us this exceptional opportunity to publish his paper.

Gios Bernardi
President of the Pezcoller Foundation
and Editor of the Pezcoller Foundation Journal

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The 2005 Pezcoller Foundations AACR award lecture
Anaheim, California, April 17, 2005

The role of Phosphoinositide 3-Kinase in Cancers

Lewis C. Cantley - Harvard Medical School

I. Discovery of the phosphoinositide 3-kinase signaling pathway and its implication in cancer.

A. Oncogenes, Growth Factors and Phosphoinositides.

In the late 1970's and early 1980's the study of viruses that cause tumors in birds and rodents uncovered a number of virus-encoded genes that were capable of transforming non-cancerous cells into cancer cells. In most cases, these cancer genes (or oncogenes) had been picked up from the host animal and incorporated into the viral genome through reverse transcription. Studies of the translational products of these oncogenes revealed a variety of proteins with intrinsic enzymatic activities, including protein-Tyr kinases ($pp60^{v\text{-}src}$, Erb-B), GTP-binding proteins (H-Ras, K-Ras) and Ser/Thr kinases (V-Raf). Although in those early days it was not at all clear how (or whether) these enzymes fit together in driving cell transformation, evidence quickly emerged that many of these proteins were components of growth factor signal transduction pathways.

At that time my laboratory was focused on determining the mechanism by which growth factors activate ion transport systems that move cations into and out of the cell. I was particularly interested in ideas originating from Robert Michell and collaborators (Michell et al., 1981) that hydrolysis of the relatively low abundant plasma membrane lipid phosphatidylinositol-4,5-bisphosphate ($PI4,5P_2$) was playing a role in regulation of calcium flux across membranes. Hydrolysis of this same lipid was also known to generate diacylglycerol, which had been shown by Takai and Nishizuki (Takai et al., 1979) to regulate a calcium-dependent protein kinase C (PKC). Thus, evidence was emerging that the phosphorylation and hydrolysis of phosphatidylinositol was playing a critical role in cellular regulation.

I was attracted to the idea that synthesis or hydrolysis of phosphoinositides at the plasma membrane in response to growth factors could be used to regulate ion transport systems or other signaling events at the plasma membrane. Plasma membrane phosphoinositides were likely to be in direct contact with ion transporters at the plasma membrane and might also mediate recruitment of cytosolic signaling proteins to the plasma membrane. There were several reports in the 1970s that growth factors stimulated ^{32}P-phosphate incorporation into plasma membrane phosphoinositides and that basal ^{32}P-phosphate incorporation into phosphoinositides was elevated in v-src transformed cells (Diringer and Friis, 1977). However, the mechanism by which growth factors or oncogenes might stimulate the synthesis or hydrolysis of phosphoinositides was obscure. Although phosphatidylinositol was known to be phosphorylated at the 4 and the 5 positions of the inositol ring to produce the two phosphoinositides phosphatidylinositol-4-phosphate (PI4P) and $PI4,5P_2$, essentially nothing was known about the kinases that carried out these reactions.

B. A phosphatidylinositol kinase co-purifies with certain activated protein-Tyr kinases

A 1983 publication from Raymond Erikson's laboratory suggested to me that there might be a direct link between protein-Tyr kinases and phosphoinositide kinases. Graziani et al., (1983) showed that in addition to the well-characterized protein-Tyr kinase activity associated with

the pp60$^{v\text{-}src}$ protein, there was also an associated glycerol kinase activity. However, glycerol was a very poor substrate with a K_M greater than 100 mM. Since pp60$^{v\text{-}src}$ was known to reside at the plasma membrane, this suggested to me that the true substrate of the glycerol kinase might be a plasma membrane lipid such as phosphatidylinositol whose inositol moiety resembled glycerol. Ian Macara, a former postdoctoral fellow from my laboratory, was visiting at that time and we debated this idea and decided it was worth testing. Ian returned to his laboratory at the University of Rochester and investigated the possibility that that the pp68$^{v\text{-}ros}$ Tyr kinase had an associated lipid kinase activity while Malcolm Whitman, a graduate student in my laboratory collaborated with Raymond Erikson's laboratory to determine whether pp60$^{v\text{-}src}$ had an associated lipid kinase activity. These studies led to the discovery that a phosphatidylinositol kinase (PI kinase) activity co-purified with both of these protein-Tyr kinases (Sugimoto et al., 1984; Macara et al., 1984). Importantly, the K_M for phosphatidylinositol was 10 mM (compared to 100 mM for glycerol) suggesting that phosphatidylinositol was more likely to be a true in vivo *substrate.*

Although initial studies suggested that the PI kinase activity and Tyr kinase activity were intrinsic properties of the same catalytic site of pp60$^{v\text{-}src}$, our later collaboration with Thomas Roberts' and Brian Schaffhausen's laboratories revealed that a similar PI kinase activity identified in the polyoma middle t/pp60$^{c\text{-}src}$ complex could be separated from the protein-Tyr kinase activity of pp60$^{c\text{-}src}$ by point mutations in middle t. (Whitman et al., 1985). More importantly, our studies of polyoma middle t mutants revealed that the transformation potential of this protein correlated with the ability to bind to PI kinase.

C. The phosphatidylinositol kinase that associates with activated Tyr kinases phosphorylates the 3 position of the inositol ring, revealing a novel phosphoinositide pathway.

Based on these results, Malcolm Whitman decided to purify and further characterize PI kinase from fibroblasts. His initial attempts at purification revealed two major PI kinases that were separable by ion exchange columns and that had very different enzymatic properties. The enzyme that had characteristics of the polyoma middle t-associated PI kinase, we named Type I and the other we named Type II (Whitman et al., 1987). In the course of characterizing these two enzymes, we noticed that the lipid products of the two enzymes migrated slightly differently on thin layer chromatography. Until this observation, we assumed that both enzymes were generating PI4P, since this was the only monophosphorylated form of phosphatidylinositol known to exist at that time. The distinct migration properties suggested that the two kinases were phosphorylating distinct sites on the inositol ring, an extremely exciting but provocative possibility.

We collaborated with Peter Downes to determine the location of the phosphorylation sites on the two lipid products and found that while the Type II enzyme phosphorylated the 4 position of the inositol ring, as expected, the Type I enzyme phosphorylated the 3 position of the ring (Whitman et al., 1988). With help from Len Stephens we were able to separate the glycerol-inositol-phosphate head groups of PI3P and PI4P by HPLC and verify that this lipid was indeed produced in vivo *(Whitman et al., 1988; Stephens et al., 1989). At this point we realized that oncogenic protein-Tyr kinases were likely regulating a novel and unexplored phosphoinositide pathway.*

D. Type I PI 3-kinase produces PI3,4P_2 and PI3,4,5P_3.

During our characterization of Type I PI kinase, we had observed that an activity that converted PI4P to a PIP_2 also co-purified with this enzyme. Leslie Serunian, a postdoctoral fellow in the laboratory analyzed this product and discovered that it was PI3,4P_2 rather than PI4,5P_2, indicating that Type I PI 3-kinase could phosphorylate the 3 position of the inositol ring whether or not the 4 position was phosphorylated.

By serendipity we also found that Type I PI 3-kinase could convert PI4,5P_2 to PI3,4,5P_3. The lab supply of PI4P was exhausted and, to our surprise, PI3,4P_2 was not produced when a new bottle of PI4P was used as substrate. When using the lipid from the new bottle, a

^{32}P-labeled spot was observed near the origin of the thin layer chromatogram. We analyzed this spot and discovered that it was PI3,4,5P$_3$. Further analysis of the contents of the new bottle labeled "PI4P" revealed that the manufacturer had miss-labeled the bottle and that it contained PI4,5P$_2$. In fact, PI4,5P$_2$ turned out to be a much better substrate of Type I PI 3-kinase than PI4P. Dr. Serunian presented this finding at a Keystone Symposium in early 1988 and we were subsequently contacted by Alexis Traynor-Kaplan and Larry Sklar informing us that they had also observed a ^{32}P-labeled lipid in neutrophils with TLC migration properties similar to those of the PI3,4,5P$_3$ that we had found (Traynor-Kaplan et al., 1988).

E. PI 3-kinase lipid products are implicated in growth factor signal transduction and in cell transformation.

Leslie Serunian in collaboration with a graduate student in the laboratory, Kurt Auger went on to investigate the presence of PI3,4P$_2$ and PI3,4,5P$_3$ in quiescent, growth factor-stimulated and oncogene-transformed cells. They found that these lipids were nominally absent in quiescent cells and appeared within less than a minute in growth factor stimulated cells (Auger et al., 1989; Serunian et al., 1990). More importantly, these lipids persisted at high levels in polyoma middle t transformed cells, even when grown at high density or in low serum (Serunian et al., 1990; Ling et al., 1992). On the basis of these results and our observation that these lipids were not substrates for PI-specific phospholipases (Serunian et al., 1989), we proposed that the lipid products of Type I PI 3-kinase (especially PI3,4P$_2$ and/or PI3,4,5P$_3$) were acting as membrane-bound second messengers to regulate cell growth and transformation (Auger et al., 1989; Cantley et al., 2001).

F. Purification of PI 3-kinase

Christopher Carpenter, a postdoctoral fellow in the laboratory, purified PI 3-kinase to homogeneity from rat liver and determined that it was a heterodimer consisting of 85 kd and 110 kd subunits (Carpenter et al., 1990). We also found that the 85 kd subunit mediated interaction with Tyr-phosphorylated proteins and could even bind to Tyr-phosphorylated middle t after renaturation from an SDS gel. Subsequently, several laboratories obtained cDNA clones of the p85 and p110 subunits, revealing multiple genes for p85 and for p110 (Escobedo et al., 1991; Skolnick et al., 1991; Hiles et al., 1992; Hu et al., 1993; reviewed by Fruman et al., 1998). The SH2 domains of the p85 subunit (revealed from the cDNA sequences) were ultimately shown to mediate the interaction with phosphoTyr residues on middle t and on receptor Tyr kinases, to explain how PI 3-kinase is recruited to activated Tyr kinases and adaptors (Cantley et al., 1991).

G. A peptide library approach reveals motifs for SH2 domains and explains recruitment of PI 3-kinase to specific activators.

In order to better understand why PI 3-kinase only bound to a minor subset of Tyr phosphorylated proteins, we developed a peptide library technique that allowed us to determine the optimal phosphoTyr peptide sequence for binding to the SH2 domains of p85 (Songyang et al., 1993). We found that the src homology 2 (SH2) domains of p85 had strong selectivities for phosphoTyr residues that were followed by the motif Met-Xxx-Met, consistent with sites on middle t and the PDGF receptor where PI 3-kinase bound (Cantley et al., 1991; Songyang et al., 1993). At that time SH2 domains were being found in a variety of oncogene products and signaling proteins. By applying our peptide library approach to a host of SH2 domains, we found that individual SH2 domains selected for phosphoTyr in unique sequence contexts, thereby explaining how relatively short sequence motifs around autophosphorylation sites of receptor Tyr kinases and adaptor proteins dictated the subsets of SH2-containing signaling proteins that were recruited and activated (Songyang et al., 1993; Songyang et al., 1994).

Further work, mostly from Michael Waterfield's laboratory, revealed that in addition to the p85/p110 type PI 3-kinases that we had originally identified (called class Ia), there were other families of PI 3-kinases in mammals (class Ib, class II and class III).

Thus far, only the class Ia enzymes have been strongly implicated in cancer. The class Ib PI 3-kinase, like the class Ia enzymes are capable of generating PI3,4P_2 and PI3,4,5P_3 in vivo *but they have a more restricted tissue distribution and are regulated by G-protein coupled receptors rather than by receptor Tyr kinases. The class III enzyme (vps34 homolog) only produces PI3P and is involved in endosome trafficking. The class II enzyme also produces PI3P and is probably involved in receptor internalization and trafficking.*

H. Lipid products of PI 3-kinase recruit PH domain containing proteins to the plasma membrane.

Although we speculated in the early 1990s that PI3,4,5P_3 and/or PI3,4P_2 were membrane embedded second messengers, at that time we did not know the targets of these lipids. A breakthrough came with the observation that certain pleckstrin homology (PH) domains were capable of binding to PI4,5P_2 (Harlan et al., 1994; Yagasawa et al., 1994). This observation led us and others to investigate the possibility that some PH domains might specifically bind to the lipid products of PI 3-kinase, ultimately leading to the discovery that the PH domain of Bruton's Tyr kinase (BTK) bound to PI3,4,5P_3 and the PH domain of an oncogene-encoded Ser/Thr kinase (AKT or PKB) bound to both PI3,4,5P_3 and PI3,4P_2 (Salim et al., 1996; Franke et al., 1997; Rameh et al., 1997; Klippel et al., 1997). These studies and subsequent studies revealed that these proteins are acutely recruited to the plasma membrane in response to activation of PI 3-kinase by growth factors (Fig. 1). At the plasma membrane, phosphorylation of these protein kinases by other resident protein kinases results in their activation, ultimately leading to downstream protein kinase signaling cascades (reviewed in Cantley, 2002). In subsequent years, additional PI-3,4,5-P_3 specific and/or PI-3,4-P_2 specific PH domain-containing proteins have been identified. These include PDK1, an upstream activator of AKT, and exchange factors for Arf and Rac family GTP-binding proteins (Karlund et al., 1997; Alessi et al., 1997; Stephens et al., 1998; Welch et al., 2003). Thus, it is now clear that the production of PI3,4P_2 and PI3,4,5P_3 in response to PI 3-kinase activation coordinates a broad spectrum of cellular events involved in cell growth, cell cycle entry, cell survival and cell migration (Cantley et al., 2002).

II. PI 3-kinase activation in human cancers

A. PTEN deletions, PIK3CA amplifications and PIK3CA mutations.

Although we discovered PI 3-kinase because of its association with tumor virus-encoded oncoproteins, and we had evidence twenty years ago that mutations in polyoma middle t that impaired binding to PI 3-kinase compromised cell transformation, the evidence that PI 3-kinase was contributing to human cancer came much later. In 1998, Jack Dixon's laboratory discovered that the PTEN tumor suppressor gene encoded a phosphatase that was capable of dephosphorylating the 3 position of PI3,4,5P_3 or PI3,4P_2 (Maehama et al., 1998). This was an important breakthrough since PTEN was known to be deleted in a large fraction of advanced human cancers, including metastatic prostate cancers, glioblastomas and melanomas. The sequence of PTEN suggested that it was a phosphotyrosine phosphatase but Dixon's laboratory found that phosphoinositides were much better substrates than phospho-proteins. Consistent with the in vitro substrate specificity of PTEN, PI3,4,5P_3 and PI3,4P_2 were found to be elevated in tumor cell lines lacking PTEN and the AKT Ser/Thr kinase was found to be constitutively activated in these cells.

Subsequent studies revealed a more direct role for the catalytic subunit of class Ia PI 3-kinase in human cancers. The gene for p110a (PIK3CA) was found to be elevated in a large fraction of ovarian cancers (Shayesteh et al., 1999) and in lung cancers (Massion et al., 2004). However, the most convincing evidence for the importance of PI 3-kinase in human cancers was the discovery that between 20 to 30% of colon and breast cancers have activating mutations in PIK3CA (Samuels et al., 2004; Bachman et al., 2004; Saal et al., 2005). Thus, loss of PTEN, amplification of PIK3CA or activating mutation in PIK3CA occur frequently in the major cancers that afflict humans (lung, colon, breast and prostate).

B. The role of PI 3-kinase in non-small cell lung carcinoma

Non-small cell lung (NSCL) carcinoma accounts for more cancer related deaths than any other human cancer. Although the vast majority of these cancers are related to smoking, a subgroup of non-smokers are afflicted with this disease. Studies over the past year have revealed that the subgroup of non-smokers are more likely to benefit from treatment with EGF receptor inhibitors (iressa/gefitinib or tarceva/erlotinib) and are more likely to have activating mutations in the EGF receptor. We investigated the activation state of the PI 3-kinase pathway in cell lines derived from NSCL tumors and found that the AKT protein-Ser/Thr kinase was activated in all the cell lines that we investigated (Engelman et al., 2005). In addition, PI 3-kinase inhibitors blocked the growth of all the NSCL carcinoma cell lines. Interestingly, we found that in a subset of the cell lines the EGF receptor inhibitor, iressa blocked activation of AKT and only in these cell lines did iressa block cell growth. These results suggested that the EGF receptor was driving activating the PI 3-kinase/AKT pathway in some NSCL tumors and that this was critical for tumor growth.

In order to understand how PI 3-kinase was activated in the NSCL carcinoma cell lines, we immunoprecipitated PI 3-kinase and used anti-phosphoTyr antibodies to visualize proteins that were bound to PI 3-kinase. We found that a ~180 kd Tyr phosphorylated protein co-immunoprecipitated with PI 3-kinase from the subset of cells that were sensitive to iressa and that addition of iressa prior to cell lysis eliminated the association with this protein. Further investigation revealed that the 180 kd protein was Erb B3, a member of the EGF receptor superfamily (Engelman et al., 2005). Erb B3 has homology to the EGF receptor and Erb B2 but lacks critical residues necessary for functional Tyr kinase activity. Thus, it acts as a substrate of the other EGF receptor Tyr kinases family members and, once phosphorylated, acts as a scaffold for recruitment and activation of PI 3-kinase (Fig. 1; see review by Carraway and Cantley, 1994). Importantly, Erb B3 is not normally expressed in lung epithelial tissue so the appearance of high levels of this protein in the iressa-sensitive tumors suggested that it played a critical role in tumor growth. Consistent with this idea, knocking down the expression of Erb B3 with siRNA eliminated the activation of AKT in the iressa-sensitive cell lines.

These results have important implications about treatment of NSCL carcinoma. They suggest that Erb B3 is a critical mediator of PI 3-kinase activation downstream of the EGF receptor in iressa-sensitive tumors. Thus, an assay for the presence of Erb B3 may be useful for identifying patients that will respond to EGF receptor inhibitors. Secondly, these results suggest that humanized monoclonal antibodies targeting Erb B3 might be useful for treating these same patients. Thirdly, these studies suggest that PI 3-kinase inhibitors might be effective on a much broader group of NSCL tumors.

III. Signaling downstream of PI 3-kinase: Tuberin, Rheb, mTOR and cell growth

A. Phosphorylation of tuberin by AKT contributes to the growth of tumor cells.

As discussed above, the Ser/Thr kinase, AKT is activated downstream of PI 3-kinase and this activation is thought to contribute to cell transformation. Investigations of PI 3-kinase and AKT mutations in flies revealed that these enzymes are in a pathway that controls cell size (reviewed in Manning and Cantley, 2003). Genetic manipulations of these genes in mice revealed that this pathway is also playing a role in cell growth regulation in mammals. In a collaboration with Seigo Izumo's laboratory, we found that transgenic expression of activated forms of PI 3-kinase p110a or of AKT in heart muscle results in enlarged hearts due to an increase in the size of cardiac myocytes (Shioi et al., 2000; 2002). More importantly, deletion of PI 3-kinase genes in heart muscle results in small hearts due to small cardiac myocytes (Luo et al., 2005).

Our first clue as to how AKT affects cell size came from our observation (in a collaboration with John Blenis' laboratory) that AKT can phosphorylate tuberin, a protein known to be involved in cell growth regulation based on drosophila genetics (Manning et al., 2002). Tuberin was originally identified as the tuberous sclerosis 2 (TSC2) gene product. Germline inactivating mutations in TSC2 result in a variety of disorders,

including mental retardation and epileptic seizures due to clusters of enlarged neuronal cells in the brain, skin hamartomas and hyperproliferation of renal epithelial tissues. Some of the characteristics of tuberous sclerosis are similar to those of Cowden's Disease, which results from germline inactivating mutations in the PTEN gene. Thus, these results suggested that both PTEN and Tuberin were negative regulators of a highly conserved pathway that controls cell growth.

Further studies from our lab and other laboratories revealed that tuberin, when in complex with a second tuberous sclerosis gene product called hamartin (TSC1) acts as a GTPase activating protein (GAP) for a ras-like GTP-binding protein called Rheb (Tee et al., 2003). By stimulating GTP hydrolysis on Rheb, the tuberin/hamartin complex keeps Rheb in an inactive state and prevents it from activating the mTOR (mammalian Targent Of Rapamycin) protein kinase (see Fig. 2). Loss of tuberin or hamartin function due to mutations or gene deletions or loss of tuberin function due to phosphorylation results in accumulation of Rheb in a GTP-bound (activated) state and leads to stimulation of mTOR. The stimulation of mTOR ultimately results in increased protein synthesis and cell growth (Fig. 2). The discovery that the TSC gene products are negative regulators of a pathway that controls mTOR was an exciting finding since it indicated that rapamycin or other mTOR inhibitors might be effective in treating this disease. On the basis of this observation, clinical trials have been initiated in which rapamycin analogs are given to TSC patients.

The elucidation of this pathway not only provided insight into the biochemical basis for tuberous sclerosis but it also suggested a mechanism by which activation of the PI 3-kinase/AKT pathway leads to cancers (Luo et al., 2003). Thus, rapamycin is being tested in human cancers where activation of PI 3-kinase is known to be a frequent event.

B. The Peutz-Jeghers Syndrome gene product (LKB1) also modulates the tuberin-Rheb-mTOR pathway.

Peutz-Jeghers syndrome (PJS) is a third inherited hamartoma syndrome with similarities to Cowden's Disease and Tuberous Sclerosis Complex. The PJS gene encodes a protein-Ser/Thr kinase called LKB1 and the mutations associated with this disease result in a non-functional kinase. In a collaboration with Ronald DePinho's laboratory we investigated the biochemical mechanism by which loss of LKB1 function results in hamartomas. We and others found that LKB1 is the upstream kinase that phosphorylates and activates AMP activated protein kinase (AMPK) (Shaw et al., 2004A). Interestingly, AMPK had been shown by Guan's laboratory to phosphorylate tuberin at distinct sites from the AKT phosphorylation sites (Inoki et al., 2003). We found that while most cell lines turn off mTOR when AMP levels become elevated, cell lines and tumors that lack LKB1 failed to turn off mTOR (Shaw et al., 2004B). These results led to the model presented in Fig. 2. Whenever cellular AMP levels become elevated due to energy stress (insufficient nutrients or oxygen), AMPK is activated (via phosphorylation by LKB1) and this results in phosphorylation of tuberin and activation of the Rheb-GAP. This eliminates mTOR activation and prevents cell growth, thereby conserving cell energy. We believe that this acts as a checkpoint to prevent small tumor masses from progressing to large aggressive tumors. However, loss of LKB1 eliminates this control mechanism and thus allows cells to continue to grow even under conditions of limited energy supply. Although many of the cells die (as is known to occur in solid tumors), some of the cells continue to grow, mutate and induce vascularization.

The model in Fig. 2 provides a biochemical explanation of why germline loss of PTEN, LKB1 or of TSC1 or TSC2 results in similar familial hamartoma syndromes. In each of these cases, Rheb accumulates in the GTP bound state and mTOR becomes activated. More importantly, sporadic loss of function of these proteins is known to occur in a large number of human cancers and it is likely that this pathway plays a central role in tumor formation. We do not yet fully understand all the components of this pathway and their mechanisms of regulation. A detailed understanding of the pathway is likely to reveal additional targets for pharmaceutical intervention and provide a logical basis of multi-drug

therapy to treat a wide variety of cancers based on knowledge of the mutations driving the disease.

IV. Summary and future perspectives:

Our understanding of the signal transduction pathways that control cell growth and cell transformation has increased dramatically during the 20 years since PI 3-kinase was first discovered. We now have detailed knowledge of how PI 3-kinase links growth factor receptors to downstream signaling pathways that regulate cell growth, cell survival, cell proliferation and cell migration. This talk has focused on control of cell growth by PI 3-kinase, yet we know that other pathways also exist for control of cell growth and cell proliferation. The signaling pathways are more complex than we could have imagined and we clearly do not yet understand all the components involved. Yet the progress that has been made in the past 20 years has already suggested new targets for pharmaceutical intervention in cancers and other diseases of cell growth regulation. It is now clear that many different combinations of mutations of oncogenes and tumor suppressor genes can give rise to a tumor. Conventional approaches for classifying tumors do not reveal the underlying mutations. Thus, the clinician treats the disease on the basis of the tissue of origin without knowledge of which biochemical pathway is driving tumor growth. However, personalized treatment for cancer is in the near future. Now that drugs are being developed to target specific components of pathways, it will be necessary for the pathologist to identify the mutations driving individual tumors in order to decide which drug combinations will be effective. The small progress that has already been made in CML and in breast and lung cancers based on this rational approach provides encouragement that we will ultimately contain this devastating disease.

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Erb B3 is a scaffold for PI3K activation downstream of the EGF receptor in NSCL carcinoma

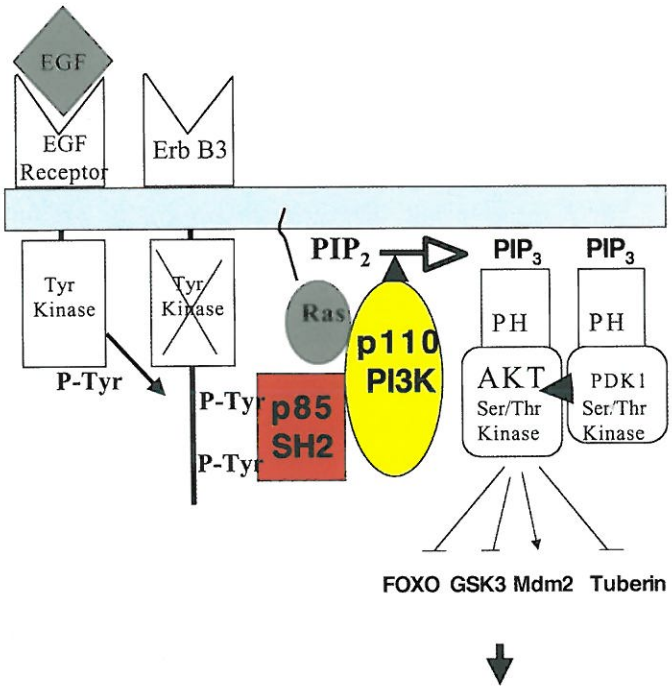

Figure 1: In cells expressing both the EGF receptor and Erb B3, activation of the EGF receptor results in tyrosine phosphorylation of Erb B3 at sites that allow binding of the SH2 domains of the p85 regulatory subunit of Class Ia PI 3-kinase. This association localizes PI 3-kinase at the plasma membrane where it phosphorylates PI4,5P$_2$ to generate PI3,4,5P$_3$. The Ser/Thr kinases, AKT and PDK1 have pleckstrin homology (PH) domains that directly bind to PI3,4,5P3 and this allows these proteins to accumulate at the plasma membrane in response to activation of PI 3-kinase. Phosphorylation of AKT by PDK1 results in activation of AKT. AKT then phosphorylates a group of proteins that modulate cell growth, cell cycle entry, cell survival and cell migration (see text).

The tuberin/hamartin complex limits cell growth by preventing activation of the mTOR protein kinase

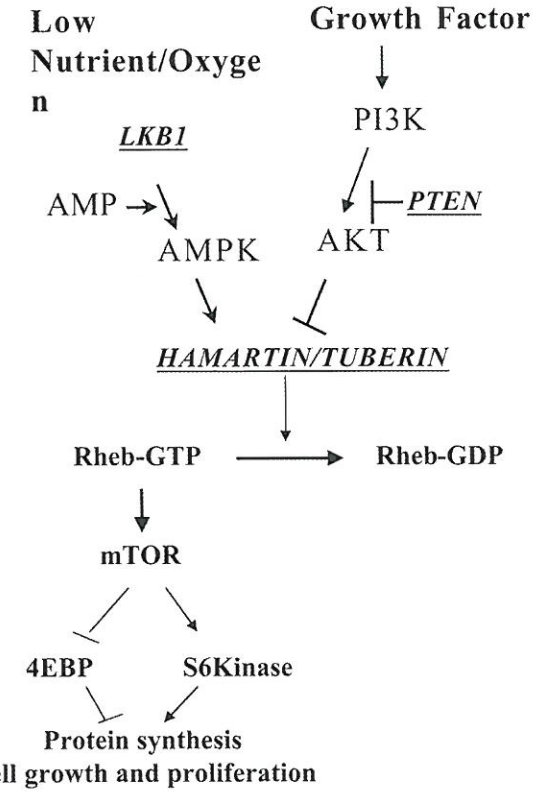

Figure 2: Tuberin in complex with hamartin acts as a GTPase activating protein for the Ras homolog RHEB, thereby turning off RHEB and preventing activation of the downstream protein kinase mTOR (mammalian target of rapamycin). Phosphorylation of tuberin by AKT in response to PI 3-kinase activation turns off the function of tuberin, allowing RHEB and mTOR to become activated. Activation of mTOR ultimately enhances translation of key proteins involved in growth control via turning off 4EBP and turning on the Ser/Thr kinase p70^{S6kinase}. When cells are low in nutrients or oxygen, AMP levels rise, resulting in activation of AMP activated protein kinase (AMPK) due to phosphorylation by the upstream kinase, LKB1. AMPK phosphorylates tuberin at distinct sites from those phosphorylated by AKT and as a consequence activates tuberin. This results in inhibition of RHEB and the mTOR pathway, thereby suppressing protein synthesis and cell growth. Loss of function of LKB1 (Peutz-Jeghers syndrome), tuberin (Tuberous Sclerosis) or PTEN (Cowden's disease) results in hyperactivation of RHEB and the mTOR pathway, resulting in hamartomas syndromes and cancers (see text).

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2007
Pezcoller Foundation-AACR International Award for Cancer Research
(€ 75.000)

The Award Criteria

The prestigious Pezcoller Foundation–AACR International Award for Cancer Research was established in 1997 to annually recognize a scientist:

- *who has made a major scientific discovery in basic cancer research or who has made significant contributions to translational cancer research;*
- *who continues to be active in cancer research and has a record of recent, noteworthy publications; and*
- *whose ongoing work holds promise for continued substantive contributions to progress in the field of cancer.*

The Award is intended to honor an individual scientist. However, more than one scientist may be co-nominated and selected to share the Award when their investigations are closely related in subject matter and have resulted in work that is worthy of the Award.

The Award consists of an unrestricted grant.

Eligibility

- *Eligible candidates are cancer researchers affiliated with institutions in academia, industry, or government that are involved in cancer research, cancer medicine, or cancer-related biomedical science anywhere in the world.*
- *Institutions or organizations are not eligible for the Award.*
- *Receipt of other major awards does not preclude a candidate from eligibility for the Award.*
- *No regard shall be given to race, gender, nationality, geographic location, or religious or political views.*

The Pezcoller Foundation was established in 1980 by Professor Alessio Pezcoller, a dedicated Italian surgeon who made important contributions to medicine during his career and who, through his foresight, vision and generous gift in support of the formation of the Foundation, stimulated others to make significant advances in cancer research. Over the past decade the Pezcoller Foundation, in collaboration with the European School of Oncology, gave a major biennial award for outstanding contributions to cancer and cancer-related biomedical science.

The American Association for Cancer Research (AACR) was founded in 1907 by eleven physicians and scientists dedicated to the conquest of cancer and now has over 24,000 members in more than 60 countries who are experts in basic, clinical, and translational cancer research. The AACR is dedicated to its mission of preventing and curing cancer through the communication of important scientific results in a variety of forums including publications, meetings and training and educational programs. Because of the commitment of the Foundation and the AACR to scientific excellence in cancer research, these organizations are now collaborating annually on the presentation of this Award.

This will strengthen international collaborations and will be a catalyst for advancements in cancer research internationally.

The winner of the Pezcoller Foundation-AACR International Award for Cancer Research will give an award lecture during the AACR Annual Meeting and will receive the award in a ceremony at the Foundation's headquarters in Trento - Italy, after the AACR annual meeting. The award consists of a prize of € 75.000 and a commemorative plaque.

Nomination Deadline: September, 2006

Questions about the nomination process should be directed to the AACR Office (Philadelphia) via Fax at (215) 440 9372- Telephone (215) 440 9300 - or E-mail awards@aacr.org

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18th Pezcoller Symposium, Trento, Italy
June 27-29, 2006

Tumor microenvironment: heterotypic interactions

Co-Chairmen:

Robert Weinberg, Whitehead Institute for Biomedical Research, Cambridge, MA; and Enrico Mihich, Roswell Park Cancer Institute, Buffalo, NY

Program Committee:

Soldano Ferrone, Raffaella Giavazzi, Douglas Hanahan, Marco Pierotti

The interactions between tumor cells and their stromal microenvironment are of increasing interest because they affect the biological behavior of cancer by influencing important processes, such as cancer progression and therefore the processes of invasion and metastasis. The symposium will be focused on these heterotypic interactions with particular emphasis on their molecular mechanisms. The roles of TGFb signaling and of urokinase and matrix-metalloproteinase in matrix remodeling, and the effects of matrix-tumor interactions on cell proliferation and migration will be discussed. The tumor-promoting effects of inflammation and of related host-cell and cytokine functions will be outlined. The signaling mechanisms that affect the biology of the stroma and the mechanisms governing angiogenesis will be debated. Finally, the clinical relevance of the phenomena discussed will be considered with emphasis on the type of intervention that may be useful toward controlling cancer. Confirmed contributors to the symposium are:
Frances Balkwill, Barts and The London Queen Mary's School of Medicine and Dentistry, London; Hartmut Beug, Research Institute of Molecular Pathology, Vienna; Francesco Blasi, Institute San Raffaele, Milan; Lisa Coussens, University of California, San Francisco; Napoleone Ferrara, Genetech, Inc, San Francisco; Soldano Ferrone, Roswell Park Cancer Institute, Buffalo; Raffaella Giavazzi, Mario Negri Institute for Pharmacological Research, Bergamo; Douglas Hanahan, University of California, San Francisco; Rakes Jain, Massachusetts General Hospital, Boston; Raghu Kalluri, Beth Israel Deaconess Medical Center, Boston; Alberto Mantovani, Istituto Clinico Humanitas, Milan; Lynn Matrisian, Vanderbilt University School of Medicine, Nashville; Harold Moses, Vanderbilt-Ingram Cancer Center, Nashville; Dario Neri, Institute of Pharmaceutical Sciences, Zurich; Marco Pierotti, Istituto Nazionale Tumori, Milan; Jeffrey Pollard, Albert Einstein College of Medicine, New York; Jacques Pouyssegur, Institute of Signaling, Developmental Biology and Cancer Research, Nice; Shahin Rafii, Weill Medical College at Cornell University, New York.

For scientific aspects of the program, contact: Enrico Mihich, MD, Roswell Park Cancer Institute, Buffalo, NY, USA, fax +716-845-8226 or 3351, e-mail: enrico.mihich@roswellpark.org
For local arrangements and administrative matters, contact Giorgio Pederzolli, Pezcoller Foundation, Trento, Italy, Fax +39-0461-980350, e-mail: pezcoller@pezcoller.it

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General Information

Travel and local Arrangements

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Via Dordi, 8 - 38100 Trento (Italy)
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www.pezcoller.it - E-mail: pezcoller@pezcoller.it

Scientific arrangements
Enrico Mihich - Roswell Park Cancer Institute
Elm & Carlton Streets, Buffalo, NY 14263 - USA
Phone 716 - 845 - 3314 - Fax 3351
E-mail: enrico.mihich@roswellpark.org

Location
The Conference will be held at Sala Palazzo Calepini
Via Calepina, 1 - 38100 Trento (Italy)
Phone 0039 - 0461 - 980250 - Fax 980350
Attendance at the meeting will be limited to a maximum of 100 participants.

Registration Fee (expenses contribution): € 200
The registration fee includes: participation to the symposium, congress kit, working lunches, coffee breaks, symposium dinner.

Posters
A limited number of poster presentations will be accepted on a competitive basis.
Pezcoller-Begnudelli Awards will be given to the 3 best posters. To submit a poster, send a one-page abstract to Dr. Mihich (by 30 April, 2005.
E-mail ann.toscani@roswellpark.org)

For this event attendance credits have been requested.

Hotel accommodations (bed and breakfast)

Hotel Trento	*€ 87,00*	*Tel. 0039 0461 271000*
Hotel Accademia	*€ 90,00*	*Tel. 0039 0461 233600*
Hotel America	*€ 60,00*	*Tel. 0039 0461 983010*
Hotel Monaco	*€ 57,00*	*Tel. 0039 0461 983060*

Messages will be taken during the Symposium at the Foundation Headquarters
Tel. 0039 0461 980250 – fax 980350

Registration Form

Tumor microenvironment: heterotypic interactions
Trento - Italy
June 27-29, 2006

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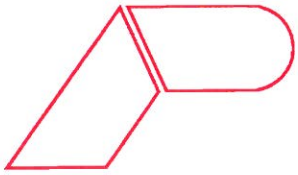

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