LABORATORY OF CELL SIGNALLING IN CANCER AND METASTASIS

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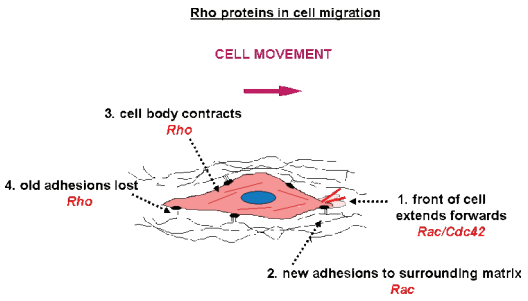
Background

Cancer is a complex disease and the understanding of deregulated signalling pathways in tumor growth is crucial. The two major aspects of cancer progression and metastasis are directional cell migration (chemotaxis) and cell survival.

Cell motility is one of the defining characteristics of invasive tumors enabling tumor cells to migrate into neighbouring tissues or to transmigrate limiting basement membranes and extracellular matrices. The invasive process is enhanced by chemotaxis of cancer cells towards extracellular signals which most often are growth factors. Clinical and experimental studies have presented evidence that link tumor progression and metastasis with tumor-associated macrophages (TAMs) and the more strong data concerns breast, prostate, ovarian and cervical cancers. Carcinoma cells of metastatic tumours chemotax to the blood vessels in response to chemoatractants such as EGF secreted by TAMs whereas cancer cells secrete CSF-1 which attracts macrophages and induces them to secrete EGF, thus completing the paracrine interaction between these two cell types by which macrophages induce tumour cell migration, invasion and intravasation. Additionally, tumor cells and macrophages appear to have a symbiotic relationship. Tumour-derived chemotactic factors recruit blood monocytes in the tumour sites where they differentiate into TAMs. Tumour cells sustain the survival of TAMs and vice versa TAMs produce factors that promote tumor growth and angiogenesis.

Rho GTPases regulate cell migration through their effects on the cytoskeleton, cell-cell and cell-substratum adhesions. It is generally accepted that Rho proteins contribute to cancer progression through their effects to migration influencing thus invasion and metastasis.

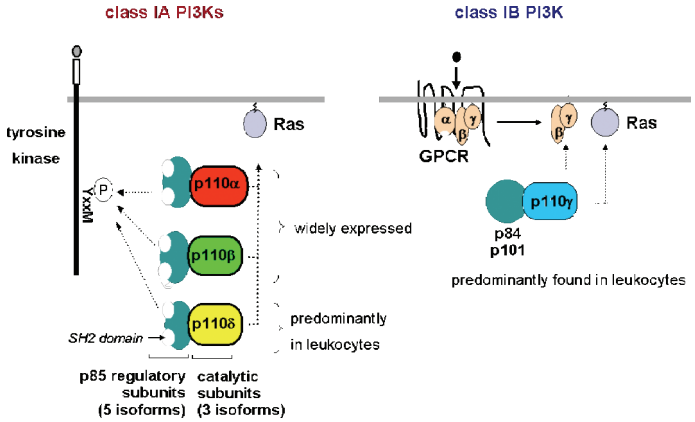


The abnormal control of cancer cells proliferation is often caused by mutation or misregulation of cell cycle-regulatory proteins or tumor suppressor proteins. Cell cycle regulation is governed by the cyclin-dependent kinases (CDKs) which when activated provide the force for the cell to move from one phase of the cell cycle to the next. The CDKs are regulated positively by cyclins and negatively by CDK inhibitors (CKIs). Expression of p27, a member of Cip/Kip family CKIs is transcriptionally controlled by Akt. Cancer cells that overexpress cyclins or do not express CKIs continue to undergo unregulated cell proliferation. However, the effect of some CKIs in cancer is almost exclusively because of their translocation from the nucleus to the cytoplasm. Akt regulates post-translationally the p21 and p27 by phosphorylating them resulting in their exclusion of the nucleus. Cytoplasmic localization of p21 and p27 is associated with high tumour grade, tumor cell invasiveness and metastasis.

The PI3K signalling pathway is now accepted as being one of the most important pathways in cell survival and proliferation. This pathway is characterized by the regulated production of the second messenger PI(3,4,5)P3 which, in turn, mediates the activation of downstream effector proteins (including Akt) leading to a plethora of important biological responses amongst them survival, growth and motility. The tumor suppressor activity of PTEN is thought to be primari-

ly due to its ability to antagonise PI3K signalling by dephosphorylating the 3-position of the inositol ring of PI(3,4,5)P3 inhibiting thus the downstream signalling. A feature of many common cancers is the misregulation of the PI(3,4,5)P3 production because of the reduced or lost activity of PTEN, the main antagonist of PI3K. Reduced activity or inactivation of PTEN creates a state in which PI3K action is uncontrolled leading to abnormal cell growth conferring thus malignant potential to the ensuing tumour.

As the PI3K pathway is important for many normal cellular functions its global inhibition could be deleterious for the organism. Therefore it is critical to understand the roles of the PI3K isoforms at the cellular and organismal level. Mammals have 8 isoforms of PI3K which have been divided in 3 classes. PI(3,4,5)P3 is produced by the class I PI3Ks, which are heterodimers made up of a 110 kDa (p110) catalytic subunit in complex with a regulatory subunit. Two subsets of class I PI3Ks have been defined. Class IA PI3Ks (p110±, p110≤ and p110¥) associate with one of 5 regulatory subunits (collectively called the 'p85s'). The class IB PI3K (p110≥) binds to a p101 or p84 subunit which has no homology to p85. All class I PI3Ks signal downstream of Ras. Class IA and IB PI3Ks are additionally activated by Tyr kinases and GPCRs, respectively.



RESEARCH

Our goal is to understand the mechanisms that regulate tumour growth and metastasis aiming to gain important information for the development of promising drugs targeting specific molecules and cell functions.
Currently, we are focusing on the pathways regulated by Rho GTPases and PI3 kinase-isoforms.

role of PI3K isoforms in the nucleus

Until recently it was thought that PI3Ks exert their effects mainly in the cytoplasm. Over the past few years, it has been shown that PI3Ks are also localized and/or translocated to the nucleus. Using cells from genetic modified mice for the PI3K isoforms we attempt to understand the individual roles that distinct PI3K isoforms play in the nucleus as well as the mechanism of PI3Ks activation in the nuclear compartment. We are also interested in understanding the mechanism that controls the translocation of p110s into the nucleus and to identify the nuclear import/export sequences in the p110s sequence.

potential link between PI3K isoforms in the nucleus with cytosolic signalling in the context of the Rho small GTPases

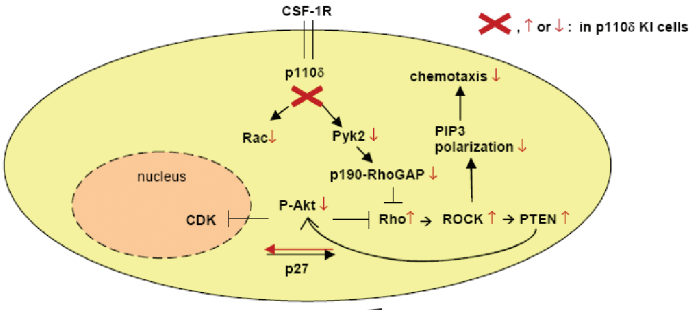
The cyclin-dependent kinase inhibitors (CKIs) when located in the nucleus function as inhibitors of cyclin-CDK activity. However, in addition to their anti-proliferative function in the

nucleus members of the Cip/Kip family (p27, p21, p57) play an oncogenic role in the cytoplasm by inhibiting the Rho GTPase's pathway at distinct points promoting thus migration.

p27 and p21 are localized in the cytoplasm after their phosphorylation by Akt a downstream effector of PI3Ks. We are investigating a potential PI3K-isoform specific regulation of cross-talk between CKIs and Rho GTPases that bridges the nuclear with cytosolic signalling. This will contribute to exploiting therapeutic strategies which by targeting one molecule will co-ordinately regulate both, cell proliferation and metastasis.

role of p110δ RhoA→PTEN feedback mechanism in cancer cells growth and metastasis

Reduce or loss-of-function of PTEN is known to lead to uncontrolled PI3K action, contributing to the increased survival, proliferation and migration observed in cancer cells. While the PTEN→PI3K pathway was generally thought to be unidirectional whereby PTEN controls PI3Ks signalling, we have discovered that this relationship is in fact bidirectional, with p110δ PI3K controlling PTEN through a feedback mechanism involving inhibition of RhoA activity.



So far the approaches to control the abnormal cancer cell growth have been focused on the inhibition of molecules downstream of PI3Ks since the inhibition of an activated signalling it's easier than the re-activation or re-introduction of a lost tumour-suppressor function. The observation that inhibition of a certain PI3K isoform can lead to enhanced activity of a key tumour suppressor was completely unexpected and opened new research fields and approaches for the control of cell survival. Using in vitro and in vivo models we are investigating the role of this pathway in tumour growth and metastasis. We are focusing on the regulation of chemotaxis, invasion and growth of tumor cells and tumor-associated macrophages and on their symbiotic relationship.

REPRESENTATIVE PUBLICATIONS

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