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Selective autophagic regulation of tumour cell viability

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Autophagy is an evolutionarily conserved catabolic membrane-trafficking process that serves to degrade long-lived proteins and is the only mechanism known for the degradation of organelles. Autophagy is therefore a major homeostatic mechanism which can degrade proteins for energy needs, or remove damaged substrates, the amino acids from which can be recycled into biosynthetic pathways. As a result, autophagy is a key regulator of cell viability and is known to promote cell survival or cell death in response to different stress conditions. In line with this, evidence indicates that autophagy may be both oncogenic and tumour suppressive during tumour development. In each context, however, autophagy is always controlled by a series evolutionarily conserved series of Atg genes. It is perceived, therefore, that specificity in the outcome of autophagy induction – i.e. whether for example to promote cell survival or death - results from upstream signalling pathways which engage the core autophagy machinery in specific ways in response to different stimuli to bring about the desired selective effect. These signalling pathways, however, remain to be defined. Here we report an RNA interference screen in Drosophila to identify kinase regulators of autophagy. Translation of the results of this screen into human cells identified the PDGFR family kinases as novel regulators of autophagy induced by hypoxia, but without impact on autophagic responses to other types of cellular stress. Hypoxia-induced autophagy is also HIF1alpha-dependent and a subsection of the HIF1-alpha transcriptome integrates convergent signals from PDGF growth factors and oxygen tension. We show that, as such, the hypoxia-selective role of PDGFR family signalling promotes adaptation to conditions of low oxygen tension and cell survival via autophagy, suggesting a pathway important in pathological states influenced by tissue hypoxia such as tumour development.

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BH3-only proteins are essential initiators of programmed cell death and stress-induced apoptosis in normal and cancer cells

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Genetic and biochemical experiments have demonstrated that BH3-only proteins are essential for initiation of programmed cell death and stress-induced apoptosis and that Bax/Bak-like proteins are required for this process, probably functioning downstream. Different BH3-only proteins are required for cell death induced by different stimuli and they can also function in a cell type-specific manner. Bim is required for the death of many cell types triggered by growth factor withdrawal, for deletion of autoreactive lymphocytes and for termination of cytotoxic T cell (CTL) immune responses. The apoptosis provoked by DNA damage requires the p53 tumor suppressor and this death is dependent on the BH3-only protein Puma and to a lesser extent also Noxa. Surprisingly, Puma was found to also be essential for apoptosis induced by several p53-independent stimuli, including cytokine withdrawal or treatment with glucocorticoids or phorbol ester. Experiments with non-transformed cells and tumour cells have demonstrated that BH3-only proteins are essential for anti-cancer therapy-induced cell killing. Puma is required for apoptosis induced by γ-radiation or several widely used chemotherapeutic drugs, including etoposide or dexamethasone. Bim, on the other hand is needed for the death of chronic myelogenous leukemia (CML) cells triggered by the BCR-ABL kinase inhibitor Gleevec.

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Dual role of autophagy in cancer

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Autophagy plays a critical protective role maintaining energy homeostasis and protein and organelle quality control. These functions are particularly important in times of metabolic stress and in cells with high energy demand such as cancer cells. In emerging cancer cells, autophagy defect may cause failure of energy homeostasis and protein and organelle quality control, leading to the accumulation of cellular damage in metabolic stress. Some manifestations of this damage, such as activation of the DNA damage response and generation of genome instability may promote tumor initiation and drive cell-autonomous tumor progression. In addition, in solid tumors, autophagy localizes to regions that are metabolically stressed. Defects in autophagy impair the survival of tumor cells in these areas, which is associated with increased cell death and inflammation. The cytokine response from inflammation may promote tumor growth and accelerate cell non-autonomous tumor progression. The overreaching theme is that autophagy protects cells from damage accumulation under conditions of metabolic stress allowing efficient tolerance and recovery from stress, and that this is a critical and novel tumor suppression mechanism. The challenge now is to define the precise aspects of autophagy, including energy homeostasis, and protein and organelle turnover, that are required for the proper management of metabolic stress that suppress tumorigenesis. Furthermore, we need to be able to identify human tumors with deficient autophagy, and to develop rational cancer therapies that take advantage of the altered metabolic state and stress responses inherent to this autophagy defect.

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SYMPOSIUM

Canceromics

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Patterns of somatic mutation in human cancer genomes

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All cancers are believed to be due to somatically acquired abnormalities in DNA. There are three major classes of somatic mutation in human cancer genomes; point mutations (including base substitutions and small insertions/deletions), rearrangements and copy number changes. Our understanding of the basic patterns and variation in pattern of these mutation types between cancers is improving as mutational screens of cancer genomes become more extensive and are conducted at higher resolution. These large scale systematic screens are yielding new cancer genes. They are also revealing traces of the mutational processes, including exposures and DNA repair defects, that have been operative during the development of individual cancers and are providing insights into the mutability of the genome itself. In this presentation recent mutational screens will be described that have provided information relating to each of these areas.

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Paradigm shift in proteomics - Implication for biomarker discovery and evaluation

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Proteomic biomarker discovery and evaluation in plasma has to overcome two major challenges: i) the vast sample complexity due to a large number of proteins and their structural diversity, and ii) the wide dynamic range of protein concentrations spanning over ten orders of magnitude. In spite of the recent technology developments, alternative strategies are still needed as classical shotgun proteomics does not provide sufficient penetration into the proteome due to undersampling issues.

We are proposing a two stage strategy, similar to the one successfully used in genomic screens: i.e. first establish a catalog of all elements (genes/proteins), and in a second stage use specific probes (proteotypic peptides) to screen for each element in multiple sample using a high throughput platform. Effective methods to reduce sample complexity together with high-performance LC-MS/MS platforms are essential.

In the initial discovery stage the isolation of a sub-proteome (N-glycosites), and its further fractionation using electro-focusing techniques is performed prior to the directed LC-MS and LC-MS/MS analyses using inclusion lists to detect, quantify, and identify biomarker candidates isolated from tissues, proximal bodily fluids, or plasma. An alternative mass spectrometry, hypothesis-driven approach, based on selected reaction monitoring (SRM) will be presented, with leverage all the existing knowledge obtained from other sources, such as transcriptomics and literature.

In the evaluation stage the candidates from the discovery phase together are used to design a mass spectrometry screen based on multiple reaction monitoring, a technique offering unique selectivity and sensitivity. This approach consists in adding peptides of interest (isotopically labeled) to the samples prior to their mass spectrometry analysis in selected monitoring mode (SRM) to ensure precise quantification, and use chromatographic (elution time) and mass spectrometric (ion intensities) properties to corroborate the identity of the analytes.

The scheduling capabilities of SRM, with its exquisite sensitivity, selectivity and dynamic range, enables analysis of multiple analytes (several hundreds) present in plasma at in sub ng/ml range. The approach was applied to several type of cancers, including ovarian cancer.