389

Regulation of genomic instability in early breast cancer

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Our recent studies on human mammary cells has allowed us to identify a previously undetected phenotype in a rare subpopulation of human mammary epithelial cells (HMEC). As previously reported, the majority of epithelial cells that grow from biopsy tissue from healthy women respond to a proliferation barrier around 15 to 20 population doublings after placement in culture. After a transient arrest (called "selection" in culture), a rare subpopulation of cells (~10-4 to 10-5) grow beyond the initial barrier and propagate for months in culture. These "post-selection" HMEC are typified by loss of specific cell cycle controls and the accumulation of a tremendous number of chromosomal abnormalities. As this population of cells is grown in culture, they approach a second growth plateau in which virtually 100% of the cells have chromosomal abnormalities. These observations challenge traditional views of how and when cells acquire genomic changes in cancer by providing a cell intrinsic mechanism that, early in the neoplastic process, generates multiple simultaneous genetic changes. These cells are generated without obligatory exposure to known physical, viral or chemical mutagenic agents. Finally, these cells possess defined characteristics that are often found in cancer cells and may explain their origin. "Post-selection" HMEC do not express p16, an important cyclin dependent kinase inhibitor, they lack proper checkpoint control and they do not maintain genomic integrity. Should these cells arise *in vivo*, they could represent the earliest steps in human mammary carcinogenesis. These observations also identify novel opportunities. They may provide potential markers for assessing susceptibility to neoplastic transformation in individuals as well as potential targets for prevention and therapy. Multiple markers clearly identify the different cellular states *in vitro* and have allowed for the identification of cells with these properties *in vivo*. Remarkably, the changes we detect in "post-selection" HMEC mimic many of the changes seen in premalignant lesions in breast cancer. We hypothesize that the above-described properties of "post-selection" HMEC *in vitro* are critically relevant to the transformation processes of mammary epithelial cells *in vivo*.

390

Genomic-stability and growth-control mechanisms involving the p38 MAP kinase signaling pathway - identification of potential targets for cancer therapy

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An intricate system of control mechanisms exists in human cells to prevent both the short-term effects, such as toxicity, and the long-term effects, such as carcinogenesis, of exposure to cytotoxic agents. Two critical control mechanisms that mediate cellular responses to such agents are the tumor suppressor p53 and G2 checkpoint controls. Recent findings indicate that p38 kinase, a subgroup of the MAP kinase signaling pathway, has a critical role in regulating both p53 and G2 cell-cycle checkpoint control. The p38 kinases are strongly and rapidly activated in most cell types by stresses such as exposure to DNA-damaging agents or activated oncogenes. p38 is an important component of the braking system that prevents the growth of such cells. p38 relays such stress signals to p53, which can cause a damaged cell to undergo apoptosis or growth arrest. Interestingly, we recently showed that the Wip1 phosphatase inhibits p38 and p53 signaling. Furthermore, we found that the gene encoding Wip1 was amplified in more that 11% of primary human breast cancers, most of which had wildtype p53. Thus, the Wip1 gene (PPM1D) behaves as a proto-oncogene in humans by blocking p38 signaling to p53 and other proteins such as Cdc25B (see below). Wip1 represents a new, attractive molecular target for anti-cancer drugs since a Wip1 inhibitor would be expected to unleash p53 in many breast cancers; this may kill the cancer cells directly and would also increase their sensitivity to cytotoxic agents used in cancer treatment such as radiation therapy. We have also recently found that p38 directly inhibits the proto-oncogene Cdc25B. The latter protein is growth stimulatory protein; Cdc25B has already been shown to function as an oncogene in some tumors. In the case of cell cycle control, p38 was found to phosphorylate regulatory sites in Cdc25B phosphatase, which is required for inhibition of this phosphatase by sequestration with 14-3-3 protein. When Cdc25B is active, it removes inhibitory phosphorylations on Cdc2 (Cdk1) and allows for the subsequent activation of Cdc2, which "drives" the cell into mitosis. After activation of p38 by stress, progression to mitosis is delayed by inhibition of Cdc25B. This allows repair of damage prior to mitosis. Evidence will be presented that inhibition of p38 can sensitize p53-deficient tumor cells to certain cytotoxic agents.

391

Defending genome integrity during S-phase: role of the Bloom's syndrome helicase

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Maintenance of genome stability is essential for cell viability and for the suppression of neoplastic transformation in mammals. The RecQ family of DNA helicases plays important roles in the suppression of chromosomal rearrangements. Recent data indicate that deficiency in RecQ helicases also leads to abnormal telomere length maintenance. In man, a defect in any of three RecQ family members predisposes affected individuals to the development of cancer. The focus of our studies is one of these cancer predisposition disorders, called Bloom's syndrome (BS). BS is a rare condition characterised by dwarfism, sunlight sensitivity, impaired fertility, and a greatly elevated incidence of leukaemias, lymphomas and solid cancers. BS is caused by loss of function mutations in the BLM gene. The hallmark of BS cells is hyperrecombination; in particular, an elevated frequency of exchanges between sister-chromatids. BS cells also demonstrate defects in DNA replication, such as a slow rate of progression through S-phase and an accumulation of abnormally-sized replication intermediates. We have purified recombinant BLM protein and shown that it is an oligomeric, ATP-dependent 3'-5' DNA helicase. Recent data indicate that BLM is an atypical helicase in being highly DNA structure-specific. In contrast to many helicases that efficiently unwind standard B-form partial duplex structures, BLM has a strong preference for alternate DNA structures such as G-quadruplex DNA, and synthetic X-junctions (4-way junctions that models the Holliday junction recombination intermediate). Consistent with a role in genetic recombination reactions, BLM can catalyze branch migration of Holliday junctions over extended distances within DNA. Moreover, BLM can form a complex with the RAD51 recombinase enzyme, and localises with RAD51 to sites of ongoing recombinational repair in cells treated with x-rays or inhibitors of DNA replication such as aphidicolin. BLM can also interact with DNA topoisomerase III and the MLH1 protein involved in DNA mismatch correction. Using purified proteins and model substrates, we are currently analysing whether BLM functionally interacts with these proteins to effect new biological roles. Our data are consistent with models in which BLM functions to prevent 'promiscuous' genetic recombination reactions that arise at sites of blocked DNA replication forks.

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Poster Sessions

Drug design and synthesis

392

Generation of novel aclacinomycin analogues by combinatorial biosynthesis to improve antitumour properties

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Aclarubicin (aclacinomycin A) was considered a promising anticancer drug because it is less cardiotoxic than doxorubicin. However, its low effectiveness on solid tumours limits its clinical use in haematological malignancies. In order to combine the favourable toxicological properties of aclarubicin and potent anticancer activity of doxorubicin, twelve novel aclacinomycin analogues were constructed by combinatorial biosynthesis. The anticancer activities *in vitro* and *in vivo* as well as the action mechanisms of the analogues were studied. A mutant strain of Streptomyces galilaeus ATCC 31615 (H075) was used as the host in combinatorial biosynthesis. H075 is deficient in biosynthesis of one of the aclacinomycin sugars, rhodinose. To alter the positions 9, 10 and 11 of the aglycone, genes from S. nogalater and S. purpurascens were introduced into H075. The novel compounds obtained were aklavinone derivatives with mono-, di- or trisaccharide moiety consisting of a rhodosamine and/or 2-deoxyfucoses.