483

In vivo antitumor activity of choline kinase inhibitors: A novel target for anticancer drug discovery

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Recent progress in deciphering the molecular basis of carcinogenesis is of utmost importance for the development of new anticancer strategies. To this end, it is essential to understand the regulation of both normal cell proliferation and its alterations in cancer cells. We have previously demonstrated that in oncogene-transformed cells, including oncogenes of relevance in human malignancies such as ras, raf and src oncogenes, there is an increased level of phosphorylcholine (PCho) resulting from a constitutive activation on choline kinase (ChoK). The importance of ChoK for the regulation of cell proliferation has also been proposed by our group, since inhibition of this enzyme drastically reduces entry into the S phase after stimulation with growth factors. Furthermore, PCho itself has mitogenic activity. We have recently reported the synthesis of compounds which are highly specific inhibitors for ChoK under ex vivo or in vitro conditions. These novel compounds also drastically reduce entry into the S phase after stimulation with specific growth factors. A more profound inhibition of cell proliferation was observed in cells transformed by oncogenes such as ras, src, raf and mos in the presence of ChoK inhibitors, compared to their parental, untransformed NIH 3T3 cells. By contrast, this effect was not observed in cells overexpressing the los oncogene. While ras, src, raf and mos transformation is associated with elevated levels of PCho, fos expression does not affect PCho levels. The inhibitory effect on proliferation of ChoK inhibitors correlates well with their ability to inhibit the production of phosphorylcholine in whole cells, a proposed novel second messenger for cell proliferation. Here we describe the characterisation of ChoK inhibitors with antiproliferative properties against human turnout-derived cell lines. The new molecules were tolerated in mice at doses that showed in vivo antitumor activity against human turnout xenografts derived from HT-29 and A431 cell lines implanted subcutaneously in nude mice. These results strongly support a critical role of choline kinase in the regulation of cell growth and makes this enzyme a novel target for the design of anticancer drugs. This first generation of inhibitors provide in vivo evidence that blockade of PCho production is a valid strategy for the development of new anticancer agents, opening a new avenue for the development of antitumor drugs with a novel mechanism of action.

484

The role of the c-erGB-4/HER4 receptor in cancer

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We have made monoclonal antibodies to the cytoplasmic domain of the human c-erbB-4 growth factor receptor. Using these to determine protein expression and in situ hybridisation to detect mRNA, we have shown that c-erbB-4 is widely expressed in normal tissues. Conversely however, expression is lower than normal in the majority of a wide range of common solid tumours, c-erbB-4 is expressed as at least four splice variants. Using PCR we have shown that the JM-a isoform is present in epithelial cells but a mixture of the cytoplasmic CTa and CTb forms are expressed, even in clonal cell lines. We have separately cloned these variants and have raised antibodies to the cytoplasmic isoforms. We plan to use these reagents to explore their function and patterns of expression in normal tissues and in cancers.

485

HER2/neu oncoprotein of human adenocarcinomas: An amplifier of signal transduction by stromal growth factor

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The erbB-2 gene (also called HER2 or neu) is overexpressed in a fraction of relatively aggressive carcinomas of the breast and other organs. Altough the encoded protein resembles several receptors for growth factors, no direct ligand of ErbB-2 has been identified. Nevertheless, ErbB-2 acts as a preferred heterodimer partner of the other three ErbB family members, and it can enhance and prolong signal transduction by many stroma-derived growth factors of the EGF and the neuregulin families. The oncogenic activity of ErbB-2 may be explained by bivalence of all ErbB ligands and the ability of ErbB-2 to act as a shared low affinity and broad selectivity receptor.

Signal transduction by ErbB receptors and the respective growth factors will be described in terms of a layered signaling network. ErbB-2 participates in the more potent routes of the network, primarily because its inactivation process (termed 'down-regulation') is impaired. Unlike ErbB-1, which is destined to intracellular degradation after ligand binding, other ErbB proteins are primarily recycled back to the cell surface. The mechanism allowing prolonged signaling by means of recycling involves an intracellular adaptor protein called c-Cbl. By binding to an activated receptor, Cbl mediates enhanced ubiquitination and subsequent degradation by lysooaml and proteasomal hydrolases. The relevance of this mechanism to immunotherapy of breast cancer will be discussed.

486

Oncogenic signalling through small GTPases

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Abnormal control of cell proliferation is a characteristic of tumours so an understanding of the mechanisms controlling cell proliferation may provide new targets for cancer therapy. Small GTPases of the Ras and Rho families are involved in transmitting signals from growth factor receptors to intracellular signalling pathways controlling cell proliferation. Furthermore mutated constitutively activated versions of these GTPases or their exchange factors can function as oncogenes. Several lines of evidence suggest that signals from Ras and Rho GTPases interact but the molecular basis for this is not fully understood. One point of interaction is that some transmembrane receptors have to activate both Ras and Rho in order to get ERK MAP kinase activation. A second point of interaction is expression of the cyclin dependent kinase inhibitor $p21^{Waf1}$. When Rho signalling is blocked activated Ras induces high levels of $p21^{Waf1}$ and fails to induce DNA synthesis.

While it is clear that activation of Ras is required for transmitting many mitogenic signals it is not clear which Ras dependent signalling pathways are required. We have addressed this problem byusing cells in which components of cell cycle control have been inactivated by homologous recombination. Using this approach we can show that loss of the tumour supressor pRb105 reduces the requirement for the ERK MAP kinase pathway for cell cycle rentry. This suggests that the ERK MAP kinase pathway plays a major role in the activation of the CyclinD dependent kinases that phosphorylate and inactivate pRb105.