H21 c-Raf is downregulated rather than required for cell survival in cells overexpressing Bcl-2

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The oncogene product Bcl-2 acts as a survival factor in a variety of cellular systems. The molecules and signalling pathways through which Bcl-2 performs its action are however ill-defined. Recently, the Ras-related protein R-Ras and c-Raf have been shown to interact with each other as well as with Bcl-2. This suggested that Bcl-2 may communicate survival signals via a Ras-Raf-dependent signal transduction pathway. However, whereas overexpression of active Raf indeed prolongs cell survival, overexpression of active R-Ras triggers cell death in a Bcl-2 suppressible manner. It is therefore unclear how Bcl-2 modulates the activities and/or signalling properties of the two proteins. Here we show that in contrast to previous findings, Bcl-2 does not interact with either Raf or R-Ras in immunoprecipitates even when the cells expressing the corresponding proteins are stressed with various apoptotic agents. Moreover, co-expression of a dominant-negative Raf mutant or cell culturing in the absence of serum reveal that Bcl-2 performs its survival action in the virtual absence of Raf activity. By contrast, Bcl-2 overexpression is associated with a drastic drop in serum-inducible Raf protein and activity levels and cells co-expressing dominant-negative Raf and Bcl-2 are even better protected from cell death than cells expressing Bcl-2 alone. These results indicate that c-Raf is not a mediator of survival signals emerging from Bcl-2, but can rather serve the role of a death signalling molecule negatively controlled by Bcl-2.

H22 CYTOKINE EXPRESSION IN PROGRESSIVE AND NON-PROGRESSIVE B-CLL

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We analysed cytokine expression by PCR and ELISA from cell and serum samples from non-progressive and progressive patients. Most samples were positive for TNFa, TGFp, INFy, IL-6 and BCGF, with a higher frequency in the non-progressive cases. We found down or up-regulation of some cytokines in activated and non activated cells. However, only TNFa and IL-10 were frequently detectable by ELISA in serum and SN from activated cells, while TGFp and INFy were undetectable both in SN and serum. IL-10 inversely correlated with disease progression and was more often detectable by ELISA in serum from non-progressive patients. IL-12 was positive by PCR only in 4/23 samples from non-progressive patients. We will further explore the significance of TNFa, IL-10 and IL-12 expression in B-CLL in relation to survival, proliferation and apoptosis.

H23 EXPRESSION OF p53 DURING NEURONAL DEATH EFFECTS OF NEOATAL TRANSECTION OF THE INFRAORBITAL NERVE ON THE CNS. Miller, Michael W., and Peter E. Kuhn, Dept. of Psychiatry, Univ. of Iowa Coll. of Med. & Veterans Affairs Med. Ctr., Iowa City IA, U.S.A.

Two candidate "death" proteins are p53 and the antigen identified by the antibody ALZ-50. Apparently, ALZ-50 recognizes a phosphorylated form of p53. We examined the expression of these proteins in the developing principal sensory nucleus of the trigeminal nerve (PSN) using both immunoblots and immunohistochemical techniques. The effect of neonatal transection of the infraorbital nerve (a major component of the trigeminal nerve) on protein expression was examined. In addition, the expression of c-fos in the developing PSN was used as an index of metabolic activity. p53- and ALZ-50-immunoreactivity peaked in the normal PSN during the first postnatal week (i.e., during the period of naturally occurring neuronal death). Transection of the infraorbital nerve directly increased the expression of the ALZ-50-positive antigen 2 hr and 2 d post-lesion, but not p53 expression. The density of p53- and ALZ-50-immunoreactive neurons was higher in the ventral ipsilateral PSN (target of the transected infraorbital nerve) than in the contralateral PSN. The density of ALZ-50-positive neurons was increased 2 hr and 2 d post-lesion, but the density of p53-labeled cells was increased only 2 d post-lesion. c-fos expression transiently rose within 2 hr of placing the lesion. Thus, the lesion initially induces the phosphorylation of available p53, and then, the upregulation of p53 synthesis. These effects (particularly the phosphorylation) occur during a catabolic phase of neuronal death, as indicated by the increase in c-fos expression.

H24 UPREGULATION OF p53 SYNTHESIS DURING NEURONAL DEATH: ASSOCIATION OF p53 WITH THE ALZ-50-POSITIVE FETAL ANTIGEN IN DEVELOPING RAT CEREBRAL CORTEX. Miller, Michael W., and Peter E. Kuhn, Dept. of Psychiatry, Univ. of Iowa Coll. of Med. & Veterans Affairs Med. Ctr., Iowa City IA, U.S.A.

Two proteins, p53 and the antigen recognized by the antibody ALZ-50, have been associated with naturally occurring neuronal death. ALZ-50 was generated against a phosphorylated protein expressed by plaques in Alzheimer's brains. We used immunoprecipitations to determine if there was a relationship between these two proteins. Crude homogenates of frontal cortex from 6-, 9-, or 12-day-old rats were immunoprecipitated with ALZ-50 or an anti-p53 antibody. After centrifugation, samples of the pellet and supernatant were immunoblotted with ALZ-50 or an anti-p53 antibody. The density of p53- and ALZ-50-immunoreactive proteins in tissue precipitated with anti-p53 was higher than in the contralateral PSN. The density of ALZ-50-positive neurons was increased 2 hr and 2 d post-lesion, but the density of p53-labeled cells was increased only 2 d post-lesion. c-fos expression transiently rose within 2 hr of placing the lesion. Thus, the lesion initially induces the phosphorylation of available p53, and then, the upregulation of p53 synthesis. These effects (particularly the phosphorylation) occur during a catabolic phase of neuronal death, as indicated by the increase in c-fos expression.