Results: The aberrant nuclear BCL10 expression was detected in six (86%) of 7 H pylori-independent high-grade gastric MALT lymphomas but none in 13 H pylori-dependent cases (P<0.001). All six patients with nuclear BCL10 expression had co-expression of nuclear NF-kB, while only two of 13 patients without nuclear BCL10 expression did so (P=0.005). Interestingly, the latter two patients were found to have tumor invasion of the gastric muscularis propria. There was a significant correlation between nuclear expression of BCL10 and NF-kB activation (P<0.001). Furthermore, the nuclear co-localization of RelA and BCL10 was confirmed by confocal immunofluorescence microscopy. The frequency of nuclear translocation of RelA was also significantly higher in H pylori-independent tumors than those H pylori-dependent tumors (6 of 7=86% versus 2 of 13=15%, P=0.004).
Conclusion: The results of this study suggest that nuclear BCL10 expression is closely associated with the nuclear NF-kB expression and support the hypothesis that nuclear BCL10 may activate NF-kB. Detection of the nuclear expression of either BCL10 or NF-kB is highly useful in the prediction of H pylori-dependent state of early-stage high-grade gastric MALT lymphoma. (This work was supported by grants from NSC91-3112-B-002-009, NHRI-91A1-CANT-1 and NTUH 91-N007)

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Identification of survivin as a transcriptional target of the Wnt pathway

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Survivin, a member of the inhibitor of apoptosis (IAP) family, is found in most cancers while undetectable in normal terminally-differentiated cells. Expressed in mitosis, survivin has been shown to inhibit apoptosis in cancer cells. Its expression is predictive of poor outcome in colorectal cancer, the second largest cause of cancer deaths in the United States. Most colorectal cancers have mutations in the Wnt pathway, where inactivation of the gatekeeper adenomatous polyposis coli (APC) inhibits its ability to bind and regulate beta-catenin. This allows abnormally high levels of beta-catenin to complex with T-cell factor (TCF) and transcriptionally activate target genes of the Wnt pathway. The objective of this study is to test a potential association between survivin and Wnt-dependent oncogenesis. Analysis of the survivin promoter yielded three potential TCF binding elements (TBE). Plasmids were constructed with increasing lengths of survivin promoter inserted upstream of a luciferase reporter gene. Promoter analysis resulted in 8 to 12-fold increased transcriptional activity in colorectal cancer cell line HCT-116, which has active Wnt signaling, as compared to HeLa cells, which have an inactive Wnt pathway. Co-transfecting plasmids containing TCF sites with beta-catenin cDNA in HCT-116 cells resulted in 2.5 to 4-fold increased transcriptional activity. Mutagenesis of each of the two most proximal TBE sites showed at least a 75% reduction in transcriptional activity compared to the wild-type construct. Transfection with a dominant-negative TCF-4 plasmid resulted in a 50% decrease in transcriptional activity as compared to control plasmid. Radioactive probes corresponding to each of the three TBE sites were shifted by nuclear HCT-116 extracts and were fully competed by molar excess of unlabeled probes in electrophoretic mobility shift assays. Survivin staining by immunohistochemistry is nearly absent in TCF-4 knockout embryonic mouse intestine. These data identify survivin as a direct transcriptional target of the Wnt/beta-catenin pathway. The overexpression of survivin in colorectal cancer via Wnt activation may contribute to modulation of apoptosis during linear step-wise tumorigenesis.

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Involvement of proapoptotic molecules Bax and Bak in TRAIL-induced apoptosis

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TRAIL/Apo-2L is a member of the tumor necrosis factor superfamily and has been demonstrated to induce apoptosis in a wide variety of cancer and transformed cells. Activation of BID, a 'BH3-domain-only' Bcl-2 family member, triggers the oligomerization of proapoptotic family members BAK or BAX, resulting in the release of cytochrome c from mitochondria to cytosol. In this study, we have shown the importance of BAX and BAK in TRAIL-induced apoptosis by studying in murine embryonic fibroblasts (MEFs) from Bax-/- Bak-/- animals. TRAIL induced cytochrome c release and apoptosis in wild type, Bid-/-, Bax-/-, or Bak-/- MEFs, but not in Bax-/- Bak-/- double knockout (DKO) MEFs. Bid, which functions upstream of cytochrome c release, was found to be cleaved in all the knock out cells except in Bid-/- MEFs. The release of cytochrome c was correlated with caspase-9 activity. TRAIL increased caspase-3 activity in all the cells except the Bax-/- Bak-/- DKO cells. TRAIL-induced mitochondrial membrane potential reduction was not observed in Bid-/-, Bax-/-, and DKO MEFs suggesting that preventing a mitochondrial membrane potential reduction alone was not sufficient to prevent TRAIL-induced apoptosis. However, microinjection of cytochrome c in DKO MEFs induced apoptosis indicating events downstream of cytochrome c are intact. Together the data suggest that activation of Bax or Bak appears to be an essential gateway to mitochondrial dysfunction required for apoptosis in response to TRAIL.

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Inhibition of PI3 kinase causes cell death through a PKB dependent mechanism and growth arrest through a PKB independent mechanism

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PTEN is one of the most commonly inactivated genes in human cancer. The mechanism by which PTEN exerts its tumor-suppressive effects has been elucidated. PTEN regulates the activity of the PI3K pathway. PI3K transduces cellular signals by phosphorylating the 3' position of phosphoinositides thus generating the second messengers PIP2 and PIP3. PTEN antagonizes this signal by dephosphorylating the 3' position of PIP3 and PIP2. PIP3 and PIP2 activate downstream effectors of PI3K. One effector of PI3K in this pathway is PKB. PKB mediates a discrete set of responses including cell survival,proliferation, migration and angiogenesis, implicating the PI3K/PKB pathway in cancer development. LNCaP is a human prostate carcinoma cell line isolated from lymph node metastasis. LNCaP is commonly used as a model for human prostate cancer because the cells are hormone dependent and express PSA. LNCaP cells have a mutation in the PTEN gene and subsequent activation of PKB. Introduction of wt PTEN into LNCaP cells, or treatment with the PI3K inhibitor LY294002, causes inactivation of PKB, growth arrest and apoptosis. While treatment with LY294002 causes inactivation of PKB and apoptosis the exact mechanism of apoptosis has not been elucidated. To specifically test the hypothesis that inhibition of PKB is responsible for the LY294002-induced apoptosis, LNCaP cells expressing a constitutively active form of PKB were generated. PKB is activated by phosphorylation on ser-473 and thr-308. Mutating these residues to aspartic acid (S473D/T308D) mimicsphosphorylation and results in activation of PKB. PKB activity is also regulated by the PH domain. Deletion of the PH domain (∗PH) leads to increased activity. Combining the S473D/T308D mutant with the ∗PH mutant generates a constitutively active PKB (∗PH-PKB-DD). The ∗PH-PKB-DD form of PKB cannot be inhibited by LY294002. LNCaP cells expressing the ∗PH-PKB-DD were generated. Activity of the ∗PH-PKB-DD protein and the inability to be inhibited by LY294002 will be confirmed by western blot analysis. Apoptosis and growth arrest were measured in the LNCaP ∗PH-PKB-DD cells and compared with control cells expressing vector alone. Both cell lines were treated with LY294002 and proliferation and cell death were measured. Expressing constitutively active PKB in LNCaP cells abrogated apoptosis induced by LY294002 but had no effect on the G1 arrest. These data identify PKB as a potential critical target for cancer therapy.

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Claudin-1 induced apoptosis in breast tumor spheroids

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Members of the claudin family together with occludin are the major constituents of the tight junction complex (TJ). The human homologue of the murine CLDN1, previously called SEMP1, was identified by molecular genetic analysis, and the CLDN1 mRNA was found to be downregulated or completely lost in human breast cancer cells *in vitro*. In this study we investigated the role of CLDN1 in CLDN1-transduced MDA-MB 361 breast tumor cells in adherent 2D and suspension 3D spheroid cell cultures. Retroviral-induced CLDN1 re-expression in breast cancer cells results in plasma membrane homing of the protein and reconstitution of paracellular flux inhibition, which is not dependent upon presence of occludin protein. There was