*Shoshan, Maria* [27]

## Cisplatin induces the pro-apoptotic conformation of bak in a delta-MEKK1-dependent manner

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Cisplatin induces the pro-apoptotic conformation of the Bcl-2 family protein Bak in four out of four human melanoma cell lines, whereas an effect on the pro-apoptotic conformation of Bax was seen in only one (FM55 cells). Expression of a kinase-inactive fragment of MEKK1 (dnMEKK) efficiently blocked the modulation of Bak and cytochrome *c* release, whereas DEVDase activation and nuclear fragmentation were reduced by half. Expression of a kinase-active MEKK1 fragment (dpMEKK) was sufficient to modulate Bak in three out of four cell lines, whereas an effect on Bax was seen only in FM55 cells. In the DFW cell line, dpMEKK stimulated a similar degree of Bak modulation as cisplatin but did not induce nuclear fragmentation. Together with the knowledge that dnMEKK was insufficient to block apoptosis completely, this finding indicates that cisplatin may induce apoptosis by means of additional mechanisms. We conclude that a pro-apoptotic MEKK1 pathway is necessary for cisplatin-induced Bak modulation, and that this modulation represents one of several cisplatin-induced apoptotic mechanisms or processes.

*Shoshan, M.C.* [28]

## BAK, BAX and p53 proteins in the apoptotic response to cisplatin

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Genotoxic damage, as induced by cisplatin, has been reported to lead to upregulation of pro-apoptotic members of the Bcl-2 family. We have here examined the pro-apoptotic Bcl-2 proteins Bak and Bax in human melanoma cells treated with cisplatin. Expression of Bak was not increased by this treatment; instead, equitoxic doses of cisplatin were found to induce the pro-apoptotic conformation of Bak in all human melanoma cell lines tested, irrespective of p53 status. Unlike Bak modulation, cisplatin-mediated modulation of Bax was seen in only one of the p53 wildtype cell lines. The upstream regulation of Bak is not known. We show that expression of a kinase-inactive fragment of the stress-activated kinase MEKK1 (dnMEKK) blocks modulation of Bak and apoptosis. Activation of the downstream kinases JNK1-2, which regulate activity of transcription factor c-Jun, has been shown to be involved in cisplatin-induced apoptosis. Although dnMEKK inhibited apoptosis, JNK1-2 activation was not blocked. Conversely, expression of a kinase-active MEKK1 fragment (dpMEKK) was able to modulate Bak and to induce apoptosis in a cell-line-dependent manner. We conclude that a pro-apoptotic MEKK1 pathway is necessary for cisplatin-induced Bak modulation, and that this modulation represents one of several cisplatin-induced apoptotic mechanisms/processes.

*Sinibaldi, Dominic* [29]

## Defining a molecular fingerprint of STAT3-regulated genes associated with oncogenesis using microarray technology and novel statistical methods

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Signal transducers and activators of transcription (STATs) are latent cytoplasmic transcription factors that are involved in normal cytokine and growth factor signaling. Recent studies have shown that certain STAT family members, most notably STAT3, are constitutively activated by various oncoproteins, such as Src. Furthermore, STAT3 signaling is constitutively activated with high frequency in diverse human tumors, including breast carcinoma, head and neck squamous cell carcinoma, multiple myeloma and various leukemias. These findings indicate that downstream target genes of STAT3 contribute to malignant progression. To date a small number of genes have been shown to be regulated by STAT3, including *cylcin D1, p21WAF1, Bcl-x, Mcl-1* and c-*Myc,* which are important in cell cycle control and apoptosis. However, it is likely that additional STAT3-regulated genes participate in oncogenesis. Using Affymetrix microarray chip technology, we have defined a list of genes that are associated with STAT3 activation in cells transformed by the Src oncoprotein and in model human breast carcinoma cell lines. We identified altered gene expression attributed to STAT3 by examining multiple cell lines with different levels of STAT3 activity. The list was refined by altering the STAT3 activation status of various cell lines following several approaches, including the use of pharmacologic inhibitors of STAT3 signaling. We employed a range of statistical approaches to identify genes that most accurately correspond to STAT3 activity in the context of oncogenesis. We have defined a molecular fingerprint of candidate STAT3-regulated genes that potentially contribute to malignant progression of breast cancer.

*Sinnett, Daniel* [30]

## A detailed transcript map of the human chromosome 12p12.3 tumor suppressor locus: the usefulness of an integrative approach

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Allellic loss of chromosome 12p is a frequent event in childhood acute lymphoblastic leukemia. This region is also deleted in several other hematological malignancies and in a variety of solid tumors, suggesting the presence of a tumor suppressor gene. The chromosomal region containing this suppressor locus was narrowed down to an interval of approximately 750 kilobases delimited by *D12S98* and *D12S358.* Since no known candidate gene was found, we initiated the construction of a detailed transcription map, focusing on a contig of four overlapping BACs. We applied a strategy integrating several complementary approaches: (1) application of computer-based data-mining tools to the existing genomic sequence (750 kb) derived form the BAC contig; (2) deployment of exon amplification and expressed sequence tag resources to identify putative complementary DNAs; (3) determination of the general expression pattern by polymerase chain reaction with reverse transcription and

northern blotting and (4) comparative genomic analysis with distant vertebrate species such as *Fugu rubripes* and *Tetraodon nigroviridis*. This transcript-mapping strategy has identified 32 potential transcription units, including 2 known genes, 5 new genes, 9 Unigene entries and 16 other expressed sequence tag clusters. The region also contains five pseudogenes. The map should facilitate subsequent efforts to characterize the candidate genes. This study illustrates how the integration of genome-based approaches facilitates the identification of genes in a large interval.

*Sjögren, Helene* [31]

## Fusion of the $NH_2$-terminal domain of the bHLH protein TCF12 to TEC in extraskeletal myxoid chondrosarcoma with translocation t(9; 15)(q22; q21)

Helene Sjögren, Barbro Wedell, Jeanne M. Meis Kindblom, Lars-Gunnar Kindblom & Göran Stenman

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Extraskeletal myxoid chondrosarcomas (EMC) are characterized by recurrent t(9; 22) or t(9; 17) translocations resulting in fusions of the $NH_2$-terminal transactivation domains of EWS or TAF2N to the entire TEC protein. We report an EMC with a new translocation, t(9; 15)(q22; q21), and a third type of TEC-containing fusion gene. The chimeric transcript encodes a protein in which the first 108 amino acids of the $NH_2$ terminus of the basic helix-loop-helix (bHLH) protein TCF12 is linked to the entire TEC protein. The translocation separates the $NH_2$-terminal domain of TCF12 from the bHLH domain as well as from a potential leucine zipper domain located immediately downstream of the breakpoint. These results demonstrate that the $NH_2$-terminal transactivation domains of EWS or TAF2N are not essential for the oncogenic properties of fusion proteins in EMC, and that EWS or TAF2N may be replaced by a similar domain from a bHLH protein that presumably endows the fusion protein with similar functions.

*Sjögren, Helene* [32]

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*Smith, David I.* [33]

## Comprehensive analysis of genetic alterations in ovarian cancer

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Ovarian cancer is the leading cause of death from gynecological malignancies among women in the United States. The 5-year survival for the patients with late stage tumors is 20%, compared to 50–90% in early stage tumors. The aim of this study is to use state-of-the-art molecular technologies to better understand the biology of ovarian cancer. We used cDNA microarrays to distinguish the variation in gene expression of approximately 20,000 genes among 10 early stage (stage I/II) and 10 late stage (stage III/IV) ovarian tumors against 5 pooled normal ovarian epithelial cell brushings. Subtracted cDNA libraries of several of these tumors versus normal ovarian epithelial cell brushings were generated to identify additional genes not present on the cDNA microarrays. Calculation of average fold induction (both up and down) revealed no statistically significant increase in the number of genes showing differential expression in the late stage tumors compared to early stage, and the differentially expressed genes in both the early and late stage tumors were very similar. The loss of expression of 20 of 30 top candidate down-regulated genes was confirmed in a panel of both early and late stage tumors (15 each) by semi-quantitative RT-PCR. To complement the gene expression profiles obtained, DNA from 35 ovarian tumors of various stages/grades were used for comparative genomic hybridization (CGH) and loss of heterozygosity (LOH) studies. Gains were commonly observed on chromosomes 1, 8, 17, 19 and 20, whereas losses were mainly observed on chromosomes 4q, 5q, 13q, and 18q. 13q14.1 and 19q13.4 were two regions that showed more loss in early stage than late stage tumors. Through these analyses we are developing a molecular signature for ovarian cancer and identifying important genes involved in early stage ovarian carcinogenesis.

*Sood, Raman* [34]

## Use of experimentally constructed haplotypes in gene mapping studies of hereditary cancers

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Conversion provides several advantages for gene mapping projects of complex diseases such as cancer. The approach takes advantage of selective retention of a subset of human chromosomes within somatic cell hybrids, isolating single copies of all