knowledge increases our understanding of how TrkB could function in aggressive human cancers, and provides important insight into the functional relationship of two key transcriptional regulators of metastasis.

**46** Poster

**Myc and Mnt in lymphomagenesis**

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The aim of this work was to investigate whether Mnt is a "master" controller of Myc complexes. Myc plays a central role in controlling cell growth, proliferation, transformation and apoptosis is deregulated in over 70% of human tumors. Mnt is thought to antagonize Myc by competitively binding to its binding partner Max and repressing gene expression. Consistent with this view, knockdown of Mnt has been shown to mimic Myc over-expression (1) and conditional Mnt deletion in mice results in mammary adenocarcinoma and T-cell lymphoma (2,3).

Recent work from our laboratory using transgenic mice emphasizes the importance of Myc levels in the development of haemopoietic malignancy (4,5). We are currently evaluating the impact of loss of Mnt in these VavP-myc10 transgenic mice. Mice bearing one copy of the VavP-myc10 transgene succumb to lymphomas of monocytic origin with a median of 41 weeks (5), while those bred to have two copies develop lethal early-onset T-cell lymphomas (median 13 weeks) (5). As the level of Myc determines both tumor kinetics and phenotype, we hypothesised that a decrease in Mnt would increase the functional level of Myc. In VavP-myc10 mice we expected this to result in acceleration of tumourigenesis and a switch of phenotype to T-cell lymphomas in mice carrying only one copy of the VavP-myc10 transgene.

Surprisingly, we found that heterozygous deletion of Mnt did not advance the onset of tumourigenesis or promote T-cell lymphomagenesis in VavP-myc10 mice. As Mnt null mice are not viable, we have now crossed Vav-Cre deleter mice with mice carrying floxed Mnt alleles to inactivate Mnt in all haemopoietic cells. These mice are viable and will be used to investigate if complete loss of Mnt disturbs haemopoiesis and results in lymphomagenesis in a manner similar to Myc over-expresson.

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**47** Poster

**A novel gene downstream of Pax2 is overexpressed in Wilms' tumors and encodes for a Calcineurin A binding protein**

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Background: Wilms' tumor (WT) accounts for approximately 85% of childhood kidney cancer and occurs at a frequency of 1 in 10,000 live births. WT is a classical cancer type arising from abnormal differentiation of kidney progenitor cells. Pax2 (Paired-Box) is recognized as a critical regulator of kidney development. Pax2 expression normally attenuates as the kidney matures; however, abnormally high PAX2 levels have been observed in both WT and renal cell carcinoma. PAX2 expression in kidney cancer cells correlates with proliferation and increased invasive phenotype. Based on the above evidence, we believe that the misexpression of PAX2 and its target genes play an important role in tumor initiation and/or progression.

Methods and Results: To test this hypothesis, we screened for target genes of Pax2 by cDNA microarray in the embryonic kidney. From this, we identified a novel gene, called CnABP for Calcineurin A Binding Partner, under Pax2 regulation. In situ hybridization indicates that CnABP coexpresses with Pax2 in the condensing mesenchyme, progenitor cells of Wilms' tumor. Expression analysis by quantitative PCR indicates that CnABP is overexpressed in more than 70% of Wilms' tumors. Interestingly, in the proportion of tumors with upregulated Pax2 expression, more than 80% also overexpress CnABP. CnABP is a highly conserved protein containing a N-myristoylation signal and a Calcineurin binding motif. Functional analysis indicates that CnABP is a membrane-anchored protein that primarily promotes cell migration. Yeast-two-hybrid and immuno-precipitation identify an interaction between CnABP and Calcineurin A, the catalytic subunit of a calcium-responsive serine/threonine phosphatase. Importantly, we showed that CnABP modulates phosphatase activities of Calcineurin.

Conclusions: Recently, components of the Calcineurin complex have been implicated as signature genes for recurrent Wilms' tumor. This is in line with the evidence we presented, as CnABP is upregulated in Wilms' tumors and is shown to promote migration. Together these data identify a new promigratory protein regulated by Pax2 that potentially play a role in tumor progression.

**48** Poster

**Tissue-specific ablation of Prkar1a causes schwannomas by suppressing neurofibromatosis protein production**

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Background: Signaling events leading to Schwann cell tumor initiation have been extensively characterized, owing to their association with the well-studied inherited tumor syndromes Neurofibromatosis (NF) Types 1 and 2. Similar tumors are also observed in patients with the endocrine neoplasia syndrome, Carney Complex (CNC), which results from inactivating mutations in PRKAR1A, the gene encoding the Type 1A regulatory subunit of the cAMP-dependent protein kinase (PKA). Loss of PRKAR1A leads to enhanced PKA activity, although the pathways leading to tumorigenesis are not well characterized. Materials and methods: We previously reported that Prkar1a$^{+/-}$ mice are tumor prone, and approximately 33% of these mice develop schwannomas. Similar tumors are observed in a limited neural crest knock-out of Prkar1a (TEC3KO) with nearly 80% penetrance by 10 months. These heterogeneous neoplasms occurred either uni- or bilaterally, and were clinically characterized as genetically engineered mouse (GEM) schwannomas, grades II and III. The TEC3KO tumors were further studied for the molecular mechanisms by which PKA dysregulation may affect NF signaling. Results: At the molecular level, analysis of the TEC3KO tumors revealed almost a complete loss of both NF proteins, whereas transcript levels were increased; indicating post-transcriptional regulation. Although Erk and Akt signaling are typically increased in NF-associated tumors, we observed no activation of either of these pathways in our tumors. Furthermore, the small G-proteins Ras, Rac1, and RhoA are all known to be involved with NF signaling. In TEC3KO tumors, all three molecules showed modest increases in total protein, but only Rac1 showed significant activation. Conclusions: These data suggest that dysregulated PKA activation causes tumorigenesis via pathways that overlap but are distinct from those described in NF tumorigenesis.

**49** Poster

**Triterpenoids affect angiogenesis and prevent neoplastic progression**

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INTRODUCTION: Angiogenesis is the base for solid tumor growth and dissemination; recently anti-angiogenic drugs have begun to show promise in clinical trials. We propose to identify molecules and pathways involved in cancer progression in order to prevent neoplastic development and metastatic dissemination. Chemoprevention focuses on the primary or secondary prevention of cancer using natural or synthetic agents usually showing mild or no collateral effects.

MATERIALS: We have recently assessed the activity of novel compounds, CDDO-Me and CDDO-Im, derived from the olanoleic acid triterpenoid, that have shown a potent antiangiogenic activity at really low dosages. In vivo we performed matrigel sponge assay; on day +4 day from matrigel injection, CDDO-treated and CTRL nu/nu CD1 mice were sacrificed to measure Hb content in matrigel sponges. Then we tested CDDO analogues efficacy in Kaposi's sarcoma xenograft; after KS-Imm cells injection in C57BL mice, we administered CDDO or vehicle and monitored tumor growth. On day of sacrifice tumors were isolated and processed for morphological and IHC analysis.In vitro we evaluated HUVECs ability to organize in capillary-like structures in matrigel, in presence of CDDO analogues or vehicle. Then we treated HUVECs and quantified proliferation. By immunofluorescence we investigated whether these compounds affect NF-kB pathway in HUVECs.

RESULTS: In vivo both CDDO-Me and CDDO-Im inhibit angiogenesis in the matrigel sponge assay and KS-Imm tumor growth. In vitro they are able to prevent endothelial cells tubulogenesis when cultured on matrigel. Moreover, from immunofluorescence experiments we observed that treatment with these triterpenoids prevents NF-kB translocation into the nucleus and thereby the activation of downstream pathways. In HUVECs CDDO-Me can inhibit the activation of erk1/2 pathway after stimulation with VEGF. CDDO-Im mechanism of action is now under study.

CONCLUSIONS: Our data confirm that inflammation, angiogenesis and the microenvironment play an important role in tumor progression. Triterpenoids in our hands target both endothelial and tumor cells. The repression of the NF-kB pathway suggests anti-inflammatory effects that may also have an indirect role in angiogenesis inhibition. CDDO-Me is now assessed in the US phase I trial in humans.

**50** Poster

**Autocrine hGH upregulates VEGF-A expression and promotes tumour angiogenesis in mammary carcinoma**

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The survival and proliferation of mammalian cells depends on the delivery of nutrients and oxygen in addition to the removal of waste products through blood vessels. In order to supply the body with nutrients, new vessels develop from pre-existing blood vessels through a process known as angiogenesis. While angiogenesis is tightly regulated in normal tissues, this process is often deregulated in cancer and important in neoplastic progression.

Autocrine human growth hormone (hGH) plays a key role in oncogenic transformation and progression of mammary cell carcinoma, both in vitro and in vivo. Autocrine hGH also promotes migration, invasion and epithelio-mesenchymal transition in the mammary carcinoma cell line, MCF-7. Here we describe a role for autocrine hGH in the development of tumour angiogenesis.

Using a previously established model of autocrine hGH expression in the mammary carcinoma cell line MCF-7, we demonstrate that autocrine hGH specifically increases vascular endothelial growth factor-A (VEGF-A) mRNA and protein levels in MCF-7 cells. Autocrine hGH production in human mammary carcinoma cells stimulated human microvascular endothelial cell (HMEC-1) survival, proliferation, migration and invasion in co-culture experiments. Furthermore, hGH expression in mammary carcinoma cells significantly stimulated HMEC-1 tube formation in Matrigel. Xenograft studies in immunosuppressed mice demonstrated that autocrine hGH promotes increased tumoural expression of the angiogenic markers VEGF-A and CD31. Autocrine hGH tumours had a greater average mass (2.6-fold) and increased tumour microvessel density (2.5-fold) as determined by CD31 staining. In addition, autocrine hGH tumours had increased immunohistochemical staining for the lymphangiogenesis markers, Podoplanin (3-fold) and Flt4 (3.1-fold).

Finally, we demonstrate that HMEC-1 express endogenous levels of hGH and VEGF-A transcript and that functional antagonism of either hGH with the hGH receptor antagonist, B2036, and/or VEGF-A with the therapeutic monoclonal antibody, Bevacizumab, reduces HMEC-1 survival, proliferation and decreases VEGF-A mRNA levels. In addition, treatment of HMEC-1 with Bevacizumab and/or B2036 reduces HMEC-1 tube formation in vitro.

These studies demonstrate that autocrine hGH promotes tumour angiogenesis in mammary carcinoma, effects which are mediated in part through increased expression of VEGF-A.

**51** Poster

**The tumor suppressor CEACAM1 is a direct transcriptional target of SOX9 in colon epithelium**

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Inactivation of the transcription factor SOX9 gene in mouse intestine affects the morphology of the colon epithelium and leads to hyperplasia (Bastide P. J Cell Biol 2007). Furthermore, overexpression of SOX9 in colon carcinoma cells resulted in apoptotic cell death increase (Jay P. Cancer Res. 2005) and cell proliferation decrease. This suggests a central role for SOX9 in the renewal of the colon epithelium. Nevertheless, direct transcriptional targets of SOX9 in this tissue are still unknown. A microarray analysis identified the tumor suppressor CEACAM1 as a possible target gene of SOX9. To study the regulation of CEACAM1 expression, we used the HT29Cl.16E colonic cells modified to express, upon doxycycline treatment, wild-type SOX9 or a SOX9 mutant form that lacks the C-terminal transcription activation domain. When SOX9 expression was induced the CEACAM1 protein content, analyzed by immunoblot, increased. On the contrary, the induction of SOX9 mutant resulted in a small decrease of CEACAM1 due to a dominant negative effect of the SOX9 mutant. CEACAM1 mRNA level, measured by real-time RT-PCR, increased 2.4-fold when SOX9 was induced and decreased 0.75-fold when SOX9 mutant was induced. A SOX9 tagged green fluorescent protein (GFP-SOX9) was transfected in SW480 colonic cells and CEACAM1 expression was monitored by immunofluorescence. As expected, overexpression of GFP-SOX9 resulted in an increase of CEACAM1 staining confirming that SOX9 up-regulates expression of CEACAM1. Moreover, we observed that, in vivo, CEACAM1 expression was reduced in colon of SOX9 deficient mouse suggesting an important role for SOX9 in the transcriptional activation of the CEACAM1 gene. The SOX9 binding sequence in the human CEACAM1 promoter was identified by luciferase reporter assays. This sequence (CTCACTGggcCTTTGTT) in position -1418 to -1402 contains a SOX consensus sequence (A/TA/TCAAA/TG) in sense orientation with two mismatches followed by three nucleotides and a perfect SOX consensus sequence in antisense orientation. Chromatin immunoprecipitation analysis provided additional evidence of the binding of SOX9 to the CEACAM1 promoter. In addition, we have found that histone acyl-transferase p300 acted as a SOX9 co-activator of the CEACAM1 promoter. We conclude that the tumor suppressor CEACAM1 is the first direct target of SOX9 identified in colon epithelium and that CEACAM1 is a good candidate to mediate a part of the anti-proliferating and pro-apoptotic activity of SOX9.

**52** Poster

**Fibroblasts nemosis signals for growth arrest and a dendritic cell-like phenotype shift in human leukemia cells**

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Interactive paracrine signaling between cancer cells and their activated stroma plays an important role in tumor development. Signals from cancer cells can induce stromal fibroblast hyperproliferation associated with increased cell-cell contacts and nemosis. Fibroblast nemosis is a unique novel type of mesenchymal cell activation that leads to production of a distinct set of signaling molecules: HGF/SF, IL-1b, IL-6, IL-8, IL-11, LIF, GM-CSF and prostaglandins.

Since the growth factors and cytokines produced are associated with differentiation of hematopoietic cells, we evaluated the effect of nemosis on human leukemia cell lines. Analysis of leukemic cells was carried out after coculture with preformed fibroblast spheroids.

Nemotic fibroblasts induced a dramatic growth inhibition of those leukemia cell lines lacking expression of c-Met, whereas growth of c-Met-positive cells was unaffected. Moreover, the responding cells showed increased adherence, motility, and chemotaxis. The cell cycle of the c-Met-negative cell lines stimulated by nemosis was arrested at the G0G1 phase. Since the growth arrest was accompanied by morphological changes such as cell elongation and formation of stellate pseudopodia, cell surface phenotype was further determined by FACS. New populations with enhanced expression of CD11c, CD13, CD45RA, CD54 and CD86 were identified in the nemosis-responsive cells. Our results show that stromal fibroblast nemosis produces signals that not only stimulate cell motility and chemotaxis but also induce differentiation to a dendritic-cell-like phenotype.

We provide here the first evidence that nemosis can produce specific signaling to arrest growth and induce differentiation of human leukemia cells. Differentiation of leukemic cells into dendritic cell lineage may stimulate T-cells and influence responses of the immune system to malignancy.

**53** Poster

**Differential transcriptional profile of the Wnt pathway in sporadic colorectal cancers with and without microsatellite instability**

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