not regulate the incidence of g-radiation-induced T-cell lymphoma but is capable of modifying the aggressiveness of tumours arising during the latency period.
Material and Methods: Mice were exposed to whole-body fractionated g-irradiation at four weekly doses for the thymic lymphoma induction. The allele expression profiles in separate stroma-enriched cell fractions and purified thymocytes were analyzed by quantitative real-time RT-PCR and western blotting. DNA sequencing was performed to identify nucleotide differences. Functional analyses were performed by immunoprecipitation and co-culture assays using transfected HEK-293-T cells and purified thymocytes.
Results: In *Tlyr1c* region, only two genes (*Cd274*/*Pdc1l1*, encoding PD-L1/B7-H1 ligand, and *Jak2*) were found exhibiting differential expression between thymus stroma cells from SEG/Pas and C57BL/6J strains. The expression of both genes increase after a single dose of g-radiation and is scarcely distinguishable in T-cell lymphomas. Several polymorphisms detected in the coding sequence of *Cd274*/*Pdc1l1* were found to be functional by co-immunoprecipitation and co-culture assays.
Conclusions: Since it is known that PD1:PD-L1 interaction can modulate survival or proliferation of thymocytes through TCR signalling, and Jak2 is a key element in the induction of PD-L1 expression, we proposed that qualitative or quantitative changes of these genes may be useful as new biomarkers for T-cell lymphomas prognosis. In particular, decreasing expression of these genes in stroma may be associated with tumour aggressiveness and significantly worse prognostic. These results improve our knowledge of the molecular mechanisms triggering T-cell lymphoblastic lymphoma development while highlighting the relevance of stroma in controlling tumoor aggressiveness.

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Ligand independent assembly of purified soluble Magic Roundabout (Robo4), a tumour-specific endothelial marker

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Background: Magic roundabout (Robo4) is the fourth recently identified member of the Roundabout receptor family. Robo4 is predominantly expressed in embryonic or tumour vascular endothelium and is considered important for vascular development and as a candidate tumor endothelial marker. Much remains unknown about Robo4, however, such as its ligand, structure, and the details of its function. Therefore, in order to study the characteristics of Robo4, we have established an expression and purification method for obtaining soluble recombinant human Robo4 (hRobo4) and mouse Robo4 (mRobo4).
Material & Methods: The cDNAs encoding extracellular domains of Robo4 s were cloned into the pcDNA3.1D/V5-His-TOPO vector. These plasmids were transfected in mammalian 293F cells and soluble Robo4 s were expressed in their supernatants. And then, soluble Robo4 s were purified using nickel nitrilotriacetic acid (Ni−NTA) chromatography and gel-filtration chromatography. Purities of soluble Robo4 s were confirmed by sodium dodecyl sulfate -polyacrylamide gel electrophoresis (SDS-PAGE). To examine the ligand-independent multimerization of purified hRobo4 and mRobo4, we calculated the native molecular weight by analytical gel-filtration and Blue Native polyacrylamide gel electrophoresis (BN-PAGE).
Result: The expression of hRobo4 and mRobo4 was observed on 6 days after transfection. The peak Robo4 fraction was observed using imidazole for elution from the Ni−NTA column. A single peak was observed in the gel-filtration chromatography and fractions of the peak were collected. By SDS-PAGE and anti Robo4 western blotting, the single broad band was observed, which may be a result of glycosylation without residual contamination. Furthermore, based on analytical gel-filtration and BN-PAGE, the native molecular weight of Robo4 was calculated to be over 200 kDa, despite the molecular weights of the Robo4 monomers were 60 to 75 kDa in SDS-PAGE analysis.
Conclusion: We established an expression and purification method for hRobo4 and mRobo4. The multimerization analysis suggests that soluble Robo4 assembled into multimers in the absence of its ligands. These purified proteins will be useful in advanced studies to determine the importance of multimerization, identify the ligands, and Robo4-mediated signaling in angiogenesis, which may contribute to the development of novel vessel-targeting therapies.

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Loss of estrogen receptor in human breast cancer cells is associated with an epithelial to mesenchymal transition

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Introduction: Loss of functional estrogen receptor (ER) is central to the development of endocrine resistant breast cancer and poses a significant clinical problem. Subsequent therapeutic intervention would benefit from increased understanding of associated molecular events participating in continued proliferation. Global gene expression was analysed in breast cancer cell lines that either over-express ER (MCF7) or in which ER is constitutively (pII) or inducibly (E2) down-regulated by transfected siRNA, to identify transcriptional response to ER blockade.
Methods: Labeled cRNA transcribed from cDNA synthesised from extracted cellular RNA was hybridised to replicate low and high density gene microarrays to compare phenotypic profiles of these cell lines; chemi-luminescence or fluorescence signals were quantified with appropriate software packages. Selected differentially expressed genes were analysed by TaqMan realtime quantitative PCR.
Results: Low density array scanning highlighted several genes that discriminated MCF7 from pII; this was confirmed and extended in the high density scans. pII cells exhibited elevated transcripts encoding proteins with motility functions, most crucially metastasis, such as urokinase plasminogen activator. Reduced ER expression was associated with loss of epithelial markers such as keratin 18/19 and increased appearance of transcripts of genes typically found in cells of mesenchymal origin; vimentin, fibronectin, cadherin 1, vascular endothelial growth factor and CD68. Differential expression of these genes was confirmed by PCR analysis, which also highlighted a similarity between pII and MDA231 cells that are *de novo* ER negative. Tetracycline-induced transient ER down-regulation in E2 cells failed to elicit the changes apparent in pII cells. Pathway analysis indicated changes in genes involved in cell-cell interaction and cell motility.
Conclusions: Our observations suggest that a change from an epithelial to a more invasive mesenchymal phenotype (a phenomenon described as EMT) may be concurrent with gradual adaptation to loss of the functional capacity of the ER transcriptional pathway, leading to an aggressive estrogen independent cancer.
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Activation of p53 stimulates transcription from the MDMX P2 promoter in tumor cells

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Aims: MDMX and MDM2 are both essential regulators of p53 activity during development and tumorigenesis. The MDM2 gene is a known p53-target gene, but transcription of the MDMX gene was assumed not to be regulated by p53. However, recently we have identified a p53-binding site in the first intron of the human MDMX gene, which indeed confers p53-induced transcription from an MDMX-P2 promoter. The putative protein translated from the MDMX-P2 transcript contains 18 additional amino acids at the N-terminus.
Methods: In this study we have analyzed the basal and stress-induced expression level of the MDMX-P2 transcript compared to the MDMX P1 and total MDMX transcripts. In comparison, the expression of other p53 target genes, i.e. MDM2 and p21 has been investigated.
Results: We found a significant increase of the MDMX- P2 transcript after cisplatin treatment of p53-wild-type tumor cell lines (OAW-42, MCF-7, U2OS, LnCap) after 24 and 48 hrs. We also found induction of the MDMX-P2 transcript in several other wt-p53 tumor cell lines after treatment with p53-activating agents, such as Etoposide. In contrast, MDMX-P2 transcript levels remained unchanged in p53-null cells (SKOV-3, SAOS, PC3). A direct involvement of p53 in the activation of the MDMX-P2-promoter was shown with the use of SAOS2 cells with inducible p53 expression.
Conclusion: Our results clearly show that the increased MDMX-P2 transcript expression upon treatment with chemotherapeutics is dependent on wt-p53. Currently, we are investigating the role of the MDX-P2-protein in the regulation of the p53-activity in response to the treatment of ovarian cancer cell lines with cisplatin.