



Lincoln's Inn Fields

## Gene Expression Analysis

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Cancer development is accompanied by a loss of tissue homeostasis either due to de-regulated proliferation or reduced cell death. Signalling pathways that are aberrantly activated in human cancer can stimulate aberrant proliferation as well as support cell survival under adverse conditions such as low nutrient or oxygen levels. Changes in cell phenotype induced by oncogenic signalling also involve alterations in cellular metabolism. The aim of the work in my lab is to understand how Akt signalling affects cellular metabolic processes that contribute to cell growth, proliferation and survival.

#### Background

Akt kinases get activated in response to growth factors, cytokines and other growth promoting stimuli and are involved in the regulation of a number of cellular processes including metabolism, cell growth, proliferation and survival. Akt targets are involved in a number of cellular processes making this kinase a central component of many signalling pathways. This makes the Akt kinase a central component of many signalling pathways.

Some of the best-characterised Akt substrates are members of the FOXO family of transcription factors. Phosphorylation of FOXO proteins by Akt creates a binding site for 14-3-3 proteins causing cytoplasmic retention of the protein and results in inhibition of FOXO target genes. FOXO factors regulate the expression of genes involved in cell cycle

regulation, de-toxication and stress response and the FOXO ortholog *daf-16* regulates longevity in *C. elegans*; indeed, it has recently been confirmed that FOXO factors function as tumour suppressors in mice. Another major signalling pathway downstream of Akt involves the mammalian target of rapamycin complex I (mTORC1). mTORC1 regulates the activity of S6-kinases and 4-EBP which are involved in the regulation of protein translation.

Several oncogenic signalling pathways increase the glycolytic rate and induce a switch to aerobic glycolysis as the major energy source in cancer cells. Inhibition of oxidative phosphorylation provides cancer cells with a growth advantage under hypoxic conditions and aerobic glycolysis ensures energy production in cells with defective mitochondria, a feature of many cancer cells. However, cancer cells also need to adapt their metabolism in order to support the increased rate of macromolecule synthesis for cell growth and proliferation. Increased nutrient uptake and metabolism as well as anapleurosis are required to fulfil this metabolic demand. Importantly, specific alterations in metabolic activity found in cancer cells also provide novel targets for therapeutic intervention.

#### The role of lipid biosynthesis in the regulation of cell growth by Protein Kinase B (PKB/Akt)

Cell growth (accumulation of mass) needs to be coordinated with the metabolic processes that are required for the synthesis of macromolecules such as DNA, proteins and lipids. The PI3-kinase/Akt signalling pathway induces cell growth via activation of complex I of the target of rapamycin (TORC1). While the significance of mTORC1 dependent induction of protein biosynthesis for cell growth is well established, the contribution of lipogenesis is less well understood.

We have previously shown that Akt-dependent activation of the two rate-limiting enzymes of the fatty acid and cholesterol biosynthetic pathways, fatty acid synthase (FASN) and HMG-CoA synthase, requires SREBP and that Akt induces nuclear accumulation of mature SREBP1 (Porstmann *et al.*, Oncogene 2005). We were subsequently able to show

that Akt induces rapid accumulation of mature SREBP1 in the nucleus. Activation of SREBP by Akt was blocked by the mTORC1 inhibitor rapamycin and by ablating mTOR expression using siRNA. We could also show that silencing of SREBP attenuates Akt-dependent lipogenesis and limits the increase in cell size in response to Akt activation in RPE cells *in vitro*. More importantly, *in vivo* silencing of dSREBP in flies caused a reduction in cell and organ size (Figure 1) and activation of dSREBP was required for the induction of cell growth by dPI3K (Porstmann *et al.*, Cell Metabolism 2008). These findings place the SREBP transcription factor downstream of a pathway that integrates growth factor signalling and nutrient availability and suggest that the PI3K/Akt/TOR pathway regulates protein and lipid biosynthesis in an orchestrated manner during cell growth.

#### Regulation of gene expression by FOXO3a

We have analysed the transcriptional programme induced by activation of FOXO3a in a colon cancer cell line (DLD1). Among the genes induced by FOXO3a were members of the Myc/Max/Mxd network of transcriptional regulators. We could show that FOXO3a induces expression of all four Mad/Mxd proteins, Mad1, Mxi1, Mad3 and Mad4. However, only Mxi1 was found to be a direct target of FOXO3a. Interestingly, induction of Mxi1 by FOXO3a is specific to the SR $\alpha$  isoform and is mediated by a cluster of highly conserved DBEs within the first intron of the *mxil* gene. We were also able to show that Mxi1 was required for efficient repression of a number of Myc target genes by FOXO3a. Furthermore, we observed that FOXO3a activation caused a switch in promoter occupancy from Myc to Mxi1 containing complexes on two E-Box containing regions of two Myc target genes. Silencing of Mxi1 and other Mad/Mxd family members reduced the cell cycle arrest and growth inhibition in response to FOXO3a activation (Delpuech *et al.*, MCB 2007). These results demonstrate that the PI3-kinase/Akt/FOXO pathway can modulate Myc function, which could contribute to cell transformation and tumour development.

#### Identification of metabolic enzymes involved in glucose metabolism and lipid synthesis required for Akt-mediated carcinogenesis

Many cancer cells rely on glycolysis as a main source of ATP

production even under conditions in which oxygen is not limiting. This phenotype, termed 'aerobic glycolysis' or 'Warburg effect' has been identified as a feature of cancer cells. Increased glycolysis increases hypoxia tolerance of tumour cells and provides cells with metabolites for the biosynthesis of macromolecules. Many tumours show increased expression of SREBP or fatty acid synthase (FASN) and exhibit increased levels of fatty acid synthesis. Furthermore, inhibitors to FASN show selective toxicity towards cancer cells. However, the role of FASN in cancer development is not yet fully understood.

In order to gain additional insight into the role of glucose and lipid metabolism in growth and survival of cancer cells, we have generated a collection of siRNA molecules that individually target 224 metabolic enzymes involved in glucose metabolism (glycolysis, TCA cycle, pentose phosphate pathway, fatty acid and cholesterol biosynthesis). We have chosen three prostate cancer cell lines, PC3, LnCAP and DU-145 and two immortalised prostate epithelial cell lines (PrEC-LH and RWPE-1) for our initial studies. We have identified a number of interesting enzymes that are selectively required for the survival of the three prostate cancer cell lines. Further studies are required to identify the contribution of these enzymes to cancer cell survival *in vitro* and *in vivo*.

Publications listed on page 126

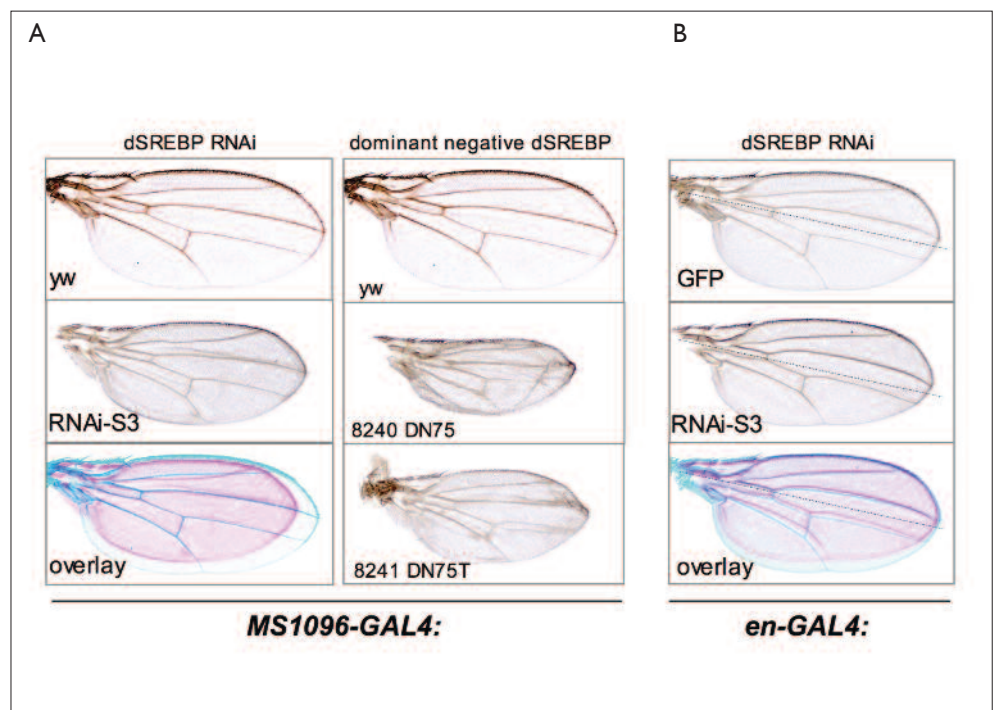


Figure 1. Inactivation of dSREBP reduces wing size in *Drosophila melanogaster*. Wing area analysis of control flies and flies expressing dSREBP RNAi or dominant negative mutants of dSREBP in different compartments of the wing. a) Inactivation of dSREBP in the dorsal cell layer of the wing causes a reduction in wing area. b) Silencing of dSREBP in the posterior compartment of the wing causes a selective reduction of this compartment.