technology is also applicable to serine/threonine kinase activity profiling we limited the analyses here to protein tyrosine kinases.
Materials and Methods: Patient derived tissues of different tumor types (e.g. breast, lung, kidney and blood) were used for extraction of total protein. Extraction involved incubation of cells or tissue in lysis buffer (6 slices of 10 um were sufficient for 20–100 microarray analyses). Equal amounts of total protein were analyzed for kinase activity on dynamic PamChip peptide microarrays. Dependent on the tissue source, amounts ranged from 1−5 ug per microarray analysis. Peptide microarrays comprised 144 or 256 different peptides. A PamChip96 plate format was used, making 96 microarray incubations in one single run possible.
Results: We show here that from all tumor tissue types tested kinase activity profiles could be generated robustly. Control experiments showed signal dependence on protein extract concentration, ATP concentration and finally, modulation of the peptide phosphorylations by kinase inhibitor drugs. Different phosphorylation profiles were obtained when the non-tumor tissue was compared to tumor tissue derived from the same patient, showing higher activities in the latter. Furthermore, different profiles were obtained that could be correlated to different patient classes.
Conclusion: A new biomarker discovery platform is presented, which is based on detection of kinase activities in protein extracts from patient derived tumor tissues by monitoring multiple peptide phosphorylation reactions on a peptide microarray. Additionally, the effect of a kinase inhibitor drug can be assessed in the same samples. This is an example of a biomarker discovery platform acting at the biological − i.e. enzymatic − level the new targeted (kinase) drugs are effective.

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CPT1C, a promising anticancer target in the treatment of hypoxic brain tumours

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Background: The tumor suppressor gene, p53, is the most commonly mutated gene in human cancers, and thus, deciphering the signaling cascade initiated by p53 is an important step towards new possibilities for novel cancer therapies.
Materials and Methods: To identify unknown genes interacting with the p53 pathway, we used the Friend virus transformed murine erythroleukemia cell line transfected with a point-mutated, temperature-sensitive p53 to perform microarray analysis. Loss- as well as gain-of-function analysis of CPT1C was performed in various human tumour cell lines. CPT1C expression was examined in 54 pediatric brain tumors by real-time RTPCR and normalized to GAPDH.
Results: Here we report that the brain-specific carnitine palmitoyl-transferase 1C (CPT1C) is a p53 target gene, induced by hypoxia and glucose deprivation in an AMPK-dependent manner. Strikingly, tumour cells engineered to constitutively express CPT1C show increased fatty acid oxidation and ATP production whereas cancer cells deficient in CPT1C function exhibit reduced ATP production, sensitivity to hypoxia and glucose deprivation. Similarly, CPT1C-deficient murine embryonic stem cells show altered fatty acid homeostasis and sensitivity to hypoxia and glucose limitation. Furthermore, tumour cells modified to constitutively express CPT1C show in vitro an increase in proliferation and migration potential, whereas tumour cells depleted of CPT1C show a decrease in proliferation and migration potential when compared to the control. Interestingly, CPT1C is upregulated in tumours and low CPT1C expression in tumours is significantly correlated with PI3 kinase pathway activation and sensitivity to rapamycin treatment in vivo whereas increased CPT1C expression confers resistance to rapamycin and glucose deprivation.
Conclusion: Our results indicate that tumour cells protect themselves against metabolic stress via CPT1C induction, perhaps by making fatty acids as an alternative fuel source. Remarkably, CPT1C depletion synergizes with metformin, an AMPK activator and suppresses tumour growth in xenograft models. CPT1C may therefore represent an exciting new therapeutic target for the treatment of hypoxic tumours.