Neue molekulare Signalwege und ihr therapeutisches Potential in der Krebsbehandlung

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PRESENTED PAPERS

1. **Zaugg K,** Suh YW, Reilly PT, Moolani Y, Cheung CC, Hakem R, Hirao A, Elledge SJ and Mak TW (2007) Crosstalk between Chk1 and Chk2 in double mutant thymocytes. **Proc Natl Acad Sci U S A**, 104(10):3805-3810.

2. **Zaugg K,** Yao Y, Reilly PT, Kannan K, Kiarash R, Mason J, Huang P, Sawyer K, Fuerth B, Faubert B, Kalliomäki T, Elia A, Luo X, Nadeem V, Bungard D, Yalavarthi S, Growney JD, Wakeham A, Moolani Y, Silvester J, You Ten A, Bakker W, Tsuchihara K, Berger SL, Hill RP, Jones RG, Tsao M, Robinson MO, Thompson CB, Pan G and Mak TW (2011) Carnitine Palmitoyltransferase 1C Promotes Cell Survival and Tumor Growth under Conditions of Metabolic Stress. **Genes Dev**, 25: 1041-1051.

3. Lohse I, Reilly P and **Zaugg K** (2011) The CPT1C 5'UTR contains a repressing upstream open reading frame that is regulated by cellular energy availability and AMPK. **PLoS ONE**. Accepted for publication May 30, 2011.

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VIII. Appendix: original papers

1. **Zaugg K et al** (2007) Crosstalk between Chk1 and Chk2 in double mutant thymocytes. **Proc Natl Acad Sci U S A**, 104(10):3805-3810.

2. **Zaugg K et al** (2011) Carnitine Palmitoyltransferase 1C Promotes Cell Survival and Tumor Growth under Conditions of Metabolic Stress. **Genes Dev**, 25: 1041-1051.

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II. Summary

Deciphering molecular pathways, which are involved in cancer development is a key step to optimize and individualize treatment against tumor cells while, reducing toxicity to normal tissue. Since the tumor suppressor p53 is the most commonly mutated or depleted gene in human cancer (Lowe et al, 2004; Vousdan et al, 2009; Chipuk and Green, 2006) my research is focussing on elucidating the signaling cascades either initiated by or upstream of p53 with the goal to identify novel molecular targets to decrease the threshold in treatment-resistant cancer cells. The tumor suppressor p53 is a transcriptional regulator with a sequence-specific DNA-binding site, thus can also be functional in a transcription-independent manner directly at the mitochondrial membrane (Moll et al., 2005). Mechanistically, p53 acts as a sensor of cellular stress (Giaccia and Kastan, 1998). Triggered by a variety of stimuli such as DNA damage and metabolic stress, the amount of p53 protein rises and the p53-dependent cascade is induced, including cell cycle arrest, DNA damage repair or cell death which determines cell fate (Lowe et al., 2004). However, how the key decision between life and death is made is not fully understood. Further elucidating these p53-induced signalling cascades will give us a novel approach to specific and successful targeting strategies against cancer.

III. Crosstalk between Chk1 and Chk2 in double mutant thymocytes (Zaugg et al, 2007, Proc Natl Acad Sci U S A)

III.1. Summary

To further elucidate the signal transduction pathway triggered by DNA damage, we focussed on Chk1, a key player in the DNA damage pathway and a kinase that activates p53 (Harris et al, 2005; Nevanlinna et al, 2006; Zhou and Elledge, 2000; Vousden et al, 2002). Since radiotherapy and many anticancer drugs are strong inducers of the DNA damage pathway, Chk1 is considered a good molecular target to increase sensitivity to cancer treatment, especially in tumour cells deficient in G1 checkpoint. But *in vitro* data show that Chk1 plays an essential role in normal tissue, especially by contributing to the maintenance of genomic stability by controlling cell division and DNA replication, which might be counteracting its function as a promising molecular target for cancer treatment (Bartek et al, 2003). Since Chk1-knockout mice are embryonic lethal, relatively little is known about its true cellular function (Liu et al, 2000; Takai et al, 2000; Lam et al, 2004). Therefore, we generated mice with a conditional deletion of Chk1 exclusively in the T-cell lineage to better understand the physiological function of Chk1 and its role in carcinogenesis. Despite the fact that Chk1 deletion is highly lethal in proliferating tissues, we succeeded by using a novel *in vivo*

phospho-flow cytometric technique to generate the first reported Chk1/Chk2 double knockout T cells. We provide evidence that the absence of Chk1 leads to cell death in early thymic development, due to an increase in apoptosis at the DN2 and DN3 stages. T-cells depleted of Chk1 showed activation of checkpoint kinase Chk2 and the tumour suppressor p53. Surprisingly, cell death in early T-cell development caused by Chk1 deletion was not rescued by p53, p21 or Brca1 inactivation.

III.2. Significance

Survival of an organism depends on the faithful transmission of genetic information from one cell to its daughter cell, which is tightly regulated by the checkpoint kinases. Therefore within recent years a lot of research has been performed to better understand the regulation and interaction of this signal transduction cascade (Carrassa, 2011). Since dysfunction or mutations of the checkpoint kinase pathways plays a crucial role in the pathogenesis of human cancers, checkpoint inhibitors seem promising anticancer agents to increase sensitivity to cancer treatment.

For the first time, we report data investigating Chk1-depleted T-cells and their genetic interaction with other genes of the checkpoint network. We and others provide strong evidence that Chk1 is critical for survival of normal proliferating tissue. These data advise caution to use Chk1 as a anticancer agent, since it would crucially influence both normal as well as tumor tissue. In fact, the Chk1 inhibitors have failed to show high level of clinical activity, most likely due to compensatory activation of other pathways like ATM/Chk2, as provided by us and other research groups (Dent et al, 2011).

IV. Carnitine Palmitoyltransferase 1C Promotes Cell Survival and Tumor Growth under Conditions of Metabolic Stress (Zaugg et al, Genes and Development, 2011)

IV.1. Summary

Tumor cells undergo metabolic adaptation in response to metabolic stress such as hypoxia and starvation to gain survival/growth advantage. Altered glycolysis is thought to be a major driver, well known over decades as the Warburg effect, but other mechanisms remain elusive (Pan and Mak, 2007).

Using a cDNA microarray screening, we identified carnitine palmitoyltransferase 1C (CPT1C) as a novel p53 target. Members of the carnitine palmitoyltransferase family play central roles in fatty acid metabolism and are localized to the mitochondrial membrane (reviewed in Bonnefont et al, 2004; Price et al, 2002; Wolfgang et al, 2006; Wolfgang et al, 2008). We show that the p53 target CPT1C is induced by hypoxia or glucose deprivation, and regulated by the metabolic sensor AMPKα. Cancer cells overexpressing CPT1C show increased

proliferation, fatty acid oxidation (FAO) and ATP production, and are resistant to glucose deprivation, hypoxia or rapamycin. Conversely, cancer cells lacking CPT1C produce less ATP and are sensitive to hypoxia or glucose deprivation. We find that murine tumors with low mTOR activation and increased rapamycin resistance also show upregulation of the normally brain-restricted CPT1C isoform of carnitine palmitoyltransferase-1.

Our results indicate that tumour cells protect themselves against metabolic stress via CPT1C induction, perhaps by making fatty acids to 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.

IV.2. Significance

In the last 20 years, cancer therapy has improved such that we see an improved prognosis for most types of cancer. This has been due to improved early-detection strategies as well as novel treatments and combination of treatment modalities. For treatment of solid tumours, however, we still lack a means to sensitize the cancer cell to one of the most important stresses upon its growth, that is survival in low oxygen microenvironment (Semenza, 2011). Hypoxic environments have the further detriment to effective treatment by virtue of the requirement of cellular oxygen for ionizing radiation-induced DNA damage, and the direct correlation between tissue oxygen concentration and accessibility of the cell to chemotherapeutics. Hence, cells in the hypoxic microenvironment are more difficult to destroy by conventional treatments (Harris, 2002).

Our study suggests that CPT1C may be therapeutically relevant. Firstly, CPT1C may be a useful biomarker for rapamycin resistance, allowing clinical selection of potential patient populations for effective treatment with mTOR inhibitors. Secondly, CPT1C may be an attractive target to affect tumors that are hypoxic and deprived of nutrients, and/or tumors that are resistant to mTOR inhibitors. Because CPT1C expression is normally brain-restricted, local administration of an agent targeting this protein would be unlikely to affect normal cells. A CPT1C inhibitor might therefore be a helpful addition to the cancer therapy armamentarium, used either as monotherapy or in combination with mTOR inhibitors.

It is our hope and expectation that, in time, effective treatments that function within the hypoxic microenvironment will be developed to allow better treatment options. At this point, CPT1C appears to be a promising target for such therapies as loss-of-function of this gene leads to enhanced hypoxia-sensitivity, thus distinguishing the target cancer cells from their properly oxygenated normal counterparts.

V. The CPT1C 5'UTR contains a repressing upstream open reading frame that is regulated by cellular energy availability and AMPK. (Lohse, Reilly, and Zaugg, PLoS ONE, 2011)

V.1. *Summary*

Translational control is an important regulator of gene expression (Morris et al, 2000). Based on our promising data on CPT1C we next investigated if posttranscriptional regulation of CPT1C might be one mean by which CPT1C's expression is modified by metabolic stress. Indeed, we are able to show that CPT1C can be regulated through an upstream open reading frame (uORF) and that this regulation can be impacted by cellular energy availability and AMPK activity. This regulation describes a heretofore unseen mechanism for metabolic control of gene expression.

V.2. Significance

The fact that regulation by the open reading frame of CPT1C is relieved in response to a specific set of stress stimuli, hints at an involvement of CPT1C in cellular energy-sensing pathways and provides further evidence for a role of the newly discovered p53 target CPT1C in metabolic stress pathways. Our studies of combining the individual derepressive stimuli would suggest either that AMPK and fatty acids may act in converging pathways for CPT1C uORF derepression, or act in a defined pathway whereby low carbohydrate concentration signals AMPK, as is known. AMPK may, in turn, then mobilize fatty acids that can then act to derepress the CPT1C uORF. Further biochemical studies will be necessary to elucidate the specific mechanism through which Palmitate and AMPK activation may control this translational derepression.

VI. Outlook

Hypoxia, or a reduction in tissue-oxygen availability, is an important chronic stress on tumour-cell growth. Cancers that have increased resistance to hypoxia are more aggressive than tumours that are sensitive. While therapeutic strategies are available that capitalize on chronic hypoxia sensitivity in tumours, primarily through intervention with angiogenesis, no cellular targets have been characterized that induce increase cellular sensitivity to hypoxia. Carnitine Palmitoyltransferase 1C (CPT1C) may represent such a target since loss-of-function of this gene promotes severe cellular sensitivity to hypoxic stress and overexpression of CPT1C leads to a significant increase of cancer cell proliferation, migration and invasion (unpublished data), the first step of cancer cells to metastases. Elucidating the mechanism by which CPT1C influences the sensitivity to hypoxia and migration might give us a novel approach to decrease the threshold in hypoxic, otherwise treatment-resistant tumour cells.

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