Network: Systems Biology of Embryonal Tumors – Neuroblastoma as Model

Project: Mechanisms of Therapy Resistance in Human Neuroblastoma

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Introduction

Neuroblastoma is a tumour derived from cells of the sympathetic nervous system and is the most common solid tumour in childhood. There are several molecular and clinical parameters that correlate with clinical outcome and are used in diagnosis. One of them is the amplification of the *MYCN* gene, which encodes a transcription factor of the Myc family of oncoproteins. Amplification of the *MYCN* oncogene discriminates two prognostic subgroups in neuroblastoma in a highly significant manner ($p=4.7 \times 10^{-24}$). Neuroblastoma patients, in whom the MYCN gene is not amplified, have an overall survival probability after a follow-up 72 months after diagnosis of a. 70%; in contrast, patients with an amplification of *MYCN* have a much lower overall survival probability of approximately 30%.

In MYCN non-amplified, age-at-diagnosis, deletion of chromosome 1 in band p36, and tumour stage are risk factors utilized in the current neuroblastoma treatment protocol (NB 2004 of the GPOH). Patients without these risk factors have a high chance of tumour regression (spontaneously or non-spontaneously). In contrast, patients that show these risk factors have a high chance of tumour progression (primary or secondary), even after complete clinical remission after intensive chemotherapy.
The molecular mechanisms underlying secondary tumour progression (relapse) after complete clinical remission with resistance even to aggressive high-dose chemotherapy regimens in patients with *MYCN* non-amplified neuroblastomas remain unexplained and are of high clinical relevance for the patients.
The aims of this project are therefore two-fold: First, we want to understand how amplification of *MYCN* affects resistance to chemotherapy and second, we want to find novel ways target that hold the potential for a more effective chemotherapy of neuroblastoma.

Results/Project Status

We have begun this project using a microarray analysis of primary human neuroblastoma tumours[1]. In this analysis, we asked whether different clinical stages of neuroblastoma could be distinguished from each other by their gene expression profiles. We also wondered whether neuroblastomas with an amplified MYCN gene differ from those with a single-copy MYCN gene. For both questions, the answer was clearly yes and we have now begun to systematically evaluate the genes that discriminate these different tumour entities to see how they contribute to tumour phenotypes.

Resistance towards retinoic acid

The expression pattern of one gene, which we termed KL1 was particularly striking, since its expression was low not only in advanced stages of neuroblastomas, but also in many of the advanced stages of other solid tumours (as judged by published expression analysis). We found that the N-Myc protein, the product of the MYCN gene, directly represses the gene encoding KL1. Strikingly, cells depleted from KL1 using siRNA are resistant to retinoic acid, one of the drugs used to treat advanced neuroblastoma. This is potentially important information, since *MYCN* amplified tumours are also know to respond poorly to this drug and our findings may provide a potential mechanism that explains these findings. Whether KL1 is also involved in regulating resistance to other chemotherapeutic agents is currently unknown and we are trying to answer this question.

Systematic search for novel genes that regulate proliferation and chemoresistance in MYCN amplified neuroblastoma

In a second part of this project, we have begun a more systematic approach to find novel genes that regulate proliferation, survival and resistance to chemotherapy in neuroblastoma. More specifically, we are interested in finding genes that are specifically required for the survival of MYCN amplified cells, but are dispensable for cells, that carry a single copy MYCN gene (as would all normal cells). Disruption of such genes would be lethal only in tumours with an amplified MYCN gene; in other words, disruption of such genes would be synthetically lethal with MYCN amplification. We believe that such genes hold great therapeutic potential as novel targets for tumour therapy.
In order to do so, we have again gone back to the list of genes that are highly expressed in MYCN amplified neuroblastoma, but not in tumour samples, which have a single copy MYCN gene. This list comprises about 200 genes. We have now cloned 3 shRNA vectors against each gene, generating a library of 600 shRNA vectors. As a first step, we are currently testing them systematically in two neuroblastoma cell lines, one with an amplified *MYCN* gene, and the other with a single copy gene. While this screen is still in progress, we have already obtained several shRNA molecules that selectively disrupt the growth of the *MYCN* amplified cells.
In order to test systematically, whether the difference between both cell lines is indeed due to enhanced activity of N-Myc, we have also established cells lines that express a conditional, hormone-inducible form of N-Myc. Several of the most promising shRNA molecules are now tested in these new cell lines.

The ubiquitin system as an Achilles' heel?

In the third part of our work, we are trying to expand work that is carried out in the lab on a related oncoprotein, (c-) Myc to the N-Myc. Both proteins are transcription actors (as mentioned above), and such proteins are traditionally considered "non-druggable", since so far it has been very difficult to control their function using small molecules.
However, it has become clear that transcription factors use enzymes for their function and are constantly being modified by enzymes and that these enzyme may be valuable drug targets. Our laboratory has recently identified one enzyme, an ubiquitin-ligase, that modifies Myc and is stringently required for transcriptional activation by Myc[2]. One approach we are taking is to determine whether this E3-ligase is also required for the function of N-Myc.
In parallel, it is also clear that proteins that are modified by ubiquitin are usually targeted to the proteasome. For some proteins it has become apparent that the action of E3-ligases, that attach ubiquitin to them, is counteract by ubiquitin-specific proteases and that these are thiol proteases that are often relatively easy to inhibit using pharmacological approaches. Inhibition of such proteases would lead to hyper-ubiquitination and enhanced degradation of the proteasome. We have obtained initial indications that indeed an ubiquitin-specific protease may exist that de-ubiquitinates Myc that is targeted for the proteasome. We are testing whether these findings extend to the N-Myc protein. If these findings indeed extend to the N-Myc protein, then

lowering the levels of N-Myc in an MYCN amplified neuroblastoma may become therapeutically feasible.

Outlook

Conventional chemotherapy of neuroblastomas using agents that are used in other tumours has done very little to improve survival changes of children with advanced neuroblastoma. It appears therefore, of paramount importance not only to understand this failure, but also to find novel target molecules that may allow a more effective therapeutic approach. We have presented here tow different strategies that, if successful, may yield such targets within the time frame of the ongoing funding period.

*Lit.: **1.** Berwanger, B. et al. Loss of a Fyn-regulated differentiation and growth arrest pathway in advanced stage neuroblastoma. Cancer Cell 2, 377-386 (2002).* **2.** *Adhikary, S. et al. The E3 ubiquitin ligase HectH9 regulates transcriptional activation by Myc and is essential for tumor cell proli*feration. Cell (in press) (2005). ***3.*** *Weber A, Imisch P, Bergmann E, Christiansen H (2004): Coamplification of DDX1 correlates with an improved survival probability in children with MYCN amplified human neuroblastoma. J Clin Oncol 22(13):2681-2690.* ***4.*** *Schramm A, Schuetz V, Christiansen H, Havers W, Papoutsi M, Wilting J, Schweigerer L (2005): High activin A expression in human neuroblastoma: suppression of malignant potential and correlation with favourable clinical outcome. Oncogene 24:680-687.* ***5.*** *Vandesompele J, Baudis M, De Preter K, Van Roy N, Ambros P, Bown N, Brinkschmidt C, Christiansen H, Combaret V, Lastowska M, Nicholson J, O´Meara A, Plantaz D, Stallings R, Brichard B, Van den Broecke C, De Bie S, De Paepe A, Laureys G, Speleman F (2005): Unequivocal delineation of clinicogenetic subgroups and development of a new model for improved outcome prediction in neuroblastoma. J Clin Oncol 23:2280-2299.* ***6.*** *Wieser R, Fritz B, Ullmann R, Muller I, Galhuber M, Storlazzi CT, Ramaswamy A, Christiansen H, Shimizu N, Rehder H (2005): Novel rearrangement of chromosome band 22q11.2 causing 22q11 microdeletion syndrome-like phenotype and rhabdoid tumor of the kidney. Hum Mutat 26: 78-83.* ***7.*** *Schramm A, Schulte JH, Klein-Hitpass L, Havers W, Sieverts H, Berwanger B, Christiansen H, Warnat P, Brors B, Eils J, Eils R, Eggert A (2005): Prediction of clinical outcome and biological characterization of neuroblastoma by expression profiling. Oncogene (Epub ahead of print).13.*