

MOLECULAR TARGETED THERAPY FOR CHILDHOOD NEUROBLASTOMA

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Amongst childhood malignancies, neuroblastoma has one of the worst survival rates, indicating an urgent need for new therapeutic approaches. Fortunately, recent advancements in the molecular genetics of neuroblastoma have enabled the identification of several prospective molecular targets that provide opportunities for the development of new therapeutic strategies. Amplification of the MYCN oncogene is a powerful predictor of poor clinical outcome in this disease. The mechanism by which MYCN amplification influences the prognosis of this disease has not been well defined. MYCN is a known transcription factor and available evidence indicates that ornithine decarboxylase (ODC1) is a critical target gene for this oncoprotein. ODC1 is the rate-limiting enzyme in the synthesis of polyamines and overexpression of this gene has been observed in a range of tumour cells, including neuroblastoma. In particular, a number of studies have provided evidence for the oncogenic and transforming abilities of ODC1. We have investigated the role of ODC1 in neuroblastoma, both in primary tumour samples and in preclinical models of the disease. In a large cohort of primary untreated neuroblastomas, Kaplan-Meier survival analysis indicated that high levels of ODC1 expression were strongly predictive of both event-free-survival and overall survival. Furthermore, a functionally important single nucleotide polymorphism within the ODC1 promoter that has been shown previously to influence gene transcription rates, was found to be significantly associated with outcome. These data suggest that inhibiting ODC1 activity would have therapeutic advantage in treating neuroblastoma. To test this hypothesis, MYCN transgenic mice that spontaneously develop neuroblastoma closely mimicking the human disease, were treated either with standard chemotherapeutic drugs alone or in combination with the ODC1 inhibitor α -difluoromethylornithine (DFMO). DFMO therapy, when combined with cytotoxic drug treatment, resulted in prolonged tumour-free survival in these mice, by comparison with the drug administered alone, in the absence of any enhanced toxicity. These findings provide strong evidence that ODC1 contributes to the malignant phenotype of neuroblastoma and suggest that targeting this oncogene for suppression of polyamine synthesis is potentially a valuable therapeutic approach for inhibiting neuroblastoma growth.

We have also shown that the aggressive drug-resistant behaviour of N-*myc*-amplified neuroblastoma is associated with N-*myc*-mediated activation of the Multidrug Resistance-associated Protein (MRP1), one of the multidrug transporters contributing to the drug resistant phenotype of neuroblastoma. In particular, we have shown high-level *MRP1* expression in primary neuroblastoma to be a powerful independent predictor of poor outcome. We have therefore chosen *MRP1* as a primary gene target for developing new prospective anti-neuroblastoma pharmaceuticals from small molecules by functional screening of chemical libraries in cell-based readout systems. Using this approach we have identified a small molecule *MRP1* inhibitor that increases the anti-tumour efficacy of both vincristine and etoposide in two murine models of neuroblastoma, which is not toxic and does not increase the toxicity associated with chemotherapeutic drug exposure. This compound appears to have high potential for the treatment of neuroblastoma and other *MRP1* over-expressing drug refractory tumours.