

The ALK-F1174L activating mutation is tumorigenic in MONC-1 neural crest stem cells in an orthotopic murine model of neuroblastoma

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Background: Activating mutations of the anaplastic lymphoma receptor tyrosine kinase gene (ALK) were identified in both somatic and familial neuroblastoma. The most common somatic mutation, F1174L, is associated with NMYC amplification and displayed an efficient transforming activity in vivo. In addition, both AKL-F1174L and NMYC were shown cooperate in neuroblastoma tumorigenesis in animal models. To analyse the role of ALK mutations in the oncogenesis of neuroblastoma, ALK wt and various ALK mutants were transduced in murine neural crest stem cells (MONC1).

Methods: ALK-wt, and F1174L, and R1275Q mutants were stably expressed by retroviral infection using the pMIGR1 vector in the murine neural crest stem cell line MONC-1, previously immortalised with v-myc, and further implanted subcutaneously or orthotopically in nude mice.

Results: Both MONC1-ALK-F1174L and -R1275Q cells displayed a rapid tumour forming capacity upon subcutaneous injection in nude mice compared to control MONC1-MIGR or MONC1 cells. Interestingly, the transforming capacity of the F1174L mutant was much more potent compared to that of R1275Q mutant in murine neural crest stem cells, while ALK-wt was not tumorigenic. In addition, mice implanted orthotopically in the left adrenal gland with MONC1-ALK-F1174L cells developed highly aggressive tumours in 100% of mice within three weeks, while MONC1-Migr or MONC1 derived tumours displayed a longer latency and a reduced tumour take.

Conclusions: The activating ALK-F1174L mutant is highly tumorigenic in neural crest stem cells. Nevertheless, we cannot exclude a functional implication of the v-myc oncogene used for MONC1 cells immortalisation. Indeed, the control MONC1-Migr and MONC1 cells were also able to derive subcutaneous and orthotopic tumours, although with considerable reduced efficiency. Further investigations using neural crest stem cell lacking exogenous myc expression are currently on way to assess the exclusive role of ALK mutations in NB oncogenesis.

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POB009 ETV5 regulates cell proliferation downstream of the ALK signaling pathway

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Background: Activating ALK mutations are present in almost 10% of primary neuroblastomas (NB) and serve as new therapeutic targets for treatment. Clinical trials for small molecule ALK inhibitors have been initiated for NB and other ALK driven tumor entities. However, in many instances, tumors acquire resistance to small molecule inhibitors, illustrating the need for additional compounds directed against downstream target genes or alternative survival pathways. To achieve this goal, we analyzed aberrant ALK signaling to identify such vulnerable nodes for combined pharmacological targeting.

Methods: Transcriptome profiling was performed on 10 NB cell lines (ALK wild type, ALK-F1174L, ALK-R1275Q mutant or amplified) following NVP-TAE684 treatment. Data mining analysis and functional validation experiments were integrated to identify ALK driven functional cellular networks and aberrantly regulated downstream pathway components.

gene signature representative for high ALK activity in NB. This signature was significantly enriched for genes implicated in MAPK/ERK signaling, including several negative MAPK regulators, indicating strong ALK induced MAPK activity. In addition, genes implicated in neuronal differentiation and growth control were identified. We selected ETV5, known to be involved in neuronal fate decision and metastasis/invasion, for further investigation. RNAi-mediated ETV5 knock down showed drastic reduction in cellular growth measured in NB cells with activated ALK. Elevated ETV5 levels were apparent in human and mouse ALK positive NB. Remarkably, inhibition of ALK signaling in NPM/ALK positive lymphoma and EML4/ALK positive lung cancer also strongly reduced ETV5 expression.

Conclusion: We obtained for the first time a detailed picture of the transcriptional consequences of sustained ALK signaling in human and mouse NB cells. The MAPK driven ETV5 oncogene was identified as a robustly regulated ALK target in NB and other ALK activated cancers, thus offering new therapeutic targets for molecular therapy.

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POB010 Screening of a natural resource library for antitumor activities using midkine as an indicator

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Background: We previously found that the growth factor midkine (MK) is highly expressed in human neuroblastoma (NB), and its blood levels work as a prognosis factor (Ikematsu et al., Cancer Sci 99, 2008; Br J Cancer 88, 2003). The purpose of this research is to search for active antitumor ingredients from marine resources, using MK expressed by NB cells as an indicator.

Methods: We used a library of 700 ingredients of marine resources extracted from the sea near Okinawa, Japan. First, ingredients of the library were added to SK-N-SH cells, a NB cell line. The culture supernatants of both 24 h and 48 h after sample addition were collected. Next, the amounts of MK in the culture supernatants were measured by MK-ELISA, which we had developed. Ingredients which showed the reduction in MK production was applied to the 2nd screening. In the 2nd screening, MK production as well as cell viability was evaluated.

Results: We obtained 71 candidates of 700 after the 1st screening. Among 71 candidates, 8 ingredients exhibited the reduction of MK production and cell viability in the 2nd screening. These ingredients were derived from marine resources classified into sponges, echinoderm, and cnidarian.

Conclusions: The ingredients with antitumor activity could be seeds for developing therapeutics for NB.

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