POB008

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

Annick Muhlethaler-Mottet, Paediatric Oncology Research, Paediatric Department, University Hospital CHUV, CH-1011 Lausanne. Switzerland; Aurélie Coulon, Paediatric Oncology Research, Paediatric Department, University Hospital CHUV, CH-1011 Lausanne. Switzerland; Katia Balmas Bourloud, Paediatric Oncology Research, Paediatric Department, University Hospital CHUV, CH-1011 Lausanne. Switzerland; Katya Nardou,Paediatric Oncology Research, Paediatric Department, University Hospital CHUV, CH-1011 Lausanne. Switzerland; Nicolas Jauquier, Paediatric Oncology Research, Paediatric Department, University Hospital CHUV, CH-1011 Lausanne. Switzerland; Jean-Marc Joseph, Paediatric Surgery, Paediatric Department, University Hospital CHUV, CH-1011 Lausanne. Switzerland; Pu Yan, Pathology Institute, University Hospital CHUV, CH-1011 Lausanne. Switzerland; Olivier Delattre, INSERM U830 Laboratoire de Génétique et Biologie des Cancers, Institut Curie, Paris Cedex 05, France; Lukas Sommer, Cell and Developmental Biology, Anatomy Institute, University of Zurich, 8057 Zurich, Switzerland; Isabelle Janoueix-Lerosey, INSERM U830 Laboratoire de Génétique et Biologie des Cancers, Institut Curie, Paris Cedex 05, France; Nicole Gross, Paediatric Oncology Research, Paediatric Department, University Hospital CHUV, CH-1011 Lausanne. Switzerland

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.

Email: Annick.Muhlethaler@chuv.ch

POB009

ETV5 regulates cell proliferation downstream of the ALK signaling pathway

Candy Kumps, Center for Medical Genetics, Ghent University Hospital, Ghent, Belgium; Irina Lambertz, Center for Medical Genetics, Ghent University Hospital, Ghent, Belgium; Michael Porcu, Center for Human Genetics, K.U.Leuven - VIB, Leuven, Belgium; Marilena De Mariano, Italian Neuroblastoma Foundation, Genoa, Italy; Translational Oncopathology,IRCCS AOU San-Martino-IST National Cancer Research Institute, Genoa, Italy; Tom Van Maerken, Center for Medical Genetics, Ghent University Hospital, Ghent, Belgium; Johannes Schulte, Department of Pediatric Oncology and Haematology, University Children's Hospital Essen,Germany; Jorge Vialard, Oncology Discovery, Janssen Research & Development, Beerse, Belgium; Jan Cools, Center for Human Genetics, K.U.Leuven - VIB, Leuven, Belgium; Katleen De Preter, Center for Medical Genetics, Ghent University Hospital, Ghent, Belgium; Frank Speleman, Center for Medical Genetics, Ghent University Hospital, Ghent, Belgium

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, ALKF1174L, ALKR1275Q 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.

Results: Differential gene expression analysis allowed delineation of a 150-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.

Email: candy.kumps@ugent.be

POB010

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

Rie SONAMOTO,OkinawaNCT/Nago,JAPAN; Chika YAMASHIRO,Bioresource engineering/Okinawa NCT/Nago,JAPAN; Karen TAKAISHI,KOUBE univ./Kobe,JAPAN; Eisuke KURAYA,OkinawaNCT/Nago,JAPAN; Shoma TSUBOTA,Dept.Biochem./Nagoya Univ.,Grad.Sch.Med./Nagoya,JAPAN; Takashi FUJIWARA,OP BIO FACTORY CO.,ltd./Uruma,JAPAN; Morita IWAMI,OP BIO FACTORY CO.,ltd./Uruma,JAPAN; Akihiko KANAMOTO,OP BIO FACTORY CO.,ltd./Uruma,JAPAN; Satoshi KISHIDA,Dept.Biochem./Nagoya Univ.,Grad.Sch.Med./Nagoya,JAPAN; Kenji KADOMATSU,Dept.Biochem./Nagoya Univ.,Grad.Sch.Med./Nagoya,JAPAN; Shinya IKEMATSU,Bioresource engineering/OkinawaNCT/Nago,JAPAN

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.

Email: ac114802@edu.okinawa-ct.ac.jp
