Original article

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Identification of a novel inherited *ALK* variant M1199L in the WNT type of medulloblastoma

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Abstract

Rearrangements involving the ALK gene were identified in a variety of cancers, including paediatric tumour neuroblastoma where presence of ALK expression is also associated with adverse prognosis. Microarrays data indicate that ALK is expressed in another paediatric tumour – medulloblastoma. Therefore, we investigated if the ALK gene is mutated in medulloblastoma and performed simultaneously the molecular profiling of tumours.

Tumours from sixty-four medulloblastoma patients were studied for detection of ALK alterations in exons 23 and 25 using Sanger method. The molecular subtypes of tumours were identified by detection of mutations in the CTNNB1 gene, monosomy 6 and by immunohistochemistry using a panel of representative antibodies.

Among three ALK variants detected two resulted in intron variants (rs3738867, rs113866835) and the third one was a novel heterozygous variant c.3595A>T in exon 23 identified in the WNT type of tumour. It resulted in methionine to leucine substitution at codon position 1199 (M1199L) of the kinase domain of ALK protein. Results of analysis using three in silico algorithms confirmed the pathogenicity of this single nucleotide variation. The same gene alteration was detected in both patient and maternal peripheral blood leukocytes indicating an inherited type of the detected variant. Presence of ALK expression in tumour tissue was confirmed by immunohistochemistry. The tumour was diagnosed as classic medulloblastoma, however with visible areas of focal anaplastic features. The patient has been disease free for 6 years since diagnosis.

This is the first evidence of an inherited ALK variant in the WNT type of medulloblastoma, what altogether with presence of ALK expression may point towards involvement of the ALK gene in this type of tumours.

Key words: *medulloblastoma, ALK gene, WNT type.*

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Introduction

The *ALK* gene encodes a tyrosine kinase receptor which is expressed in the developing nervous systems [13]. The *ALK* gene is also expressed in neuroblastoma, the most common extracranial childhood tumour originating in the sympathetic nervous system and presence of expression is associated with adverse prognosis [8,26]. Importantly, *ALK* gene defects were reported both in terms of copy number changes and presence of somatic mutations in up to 10% of tumours, with two hotspot mutations in exon 23 (F1174L) and in exon 25 (R1275Q) [1]. Also germline *ALK* mutations were identified in both sporadic and familial cases of neuroblastoma [16,21] which altogether points to *ALK* as a key oncogene associated with this disease. Distribution of *ALK* mutations may be not random but typical for a particular molecular subtype of tumour. For example, F1174L substitution occurs more frequently in tumours with *MYCN* amplification [6].

Medulloblastoma, on the other hand, is the most common malignant intracranial childhood tumour of neuronal origin. Recent molecular studies revealed the existence of at least four molecular subtypes which display distinctive profiles of gene expression: Wingless (WNT), Sonic Hedgehog (SHH), Group 3 and Group 4 tumours [3,18,22]. Microarrays data indicate that *ALK* expression is associated with the WNT group, since the *ALK* gene was identified among top 50 most highly expressed genes in WNT tumours as compared to all other groups [18, in supplementary data]. Recent next generation sequencing studies in medulloblastoma based on analyses of 'discovery sets' of 37-92 tumours did not reveal any *ALK* gene alterations [17,23,24]. However the WNT groups analysed in all of these studies were small (from 5 to 7 tumours), thus preventing from discovery of changes occurring at low frequency. In support of that, the first *ALK* germline variant (3605delG) was discovered recently in a medulloblastoma patient pointing to a possible role of this gene in medulloblastoma [5].

Therefore, we further analysed exons 23 and 25 of the *ALK* gene together with molecular characteristics of tumours and found the second novel inherited *ALK* likely pathogenic variant in the WNT subtype of medulloblastoma.

Material and methods

Patients and tumour material

Sixty-four medulloblastoma patients treated from 1999 to 2014 in the Children's Memorial Health Institute (CMHI) in Warsaw, Poland were included in the analysis.

Analysis was performed on frozen and formalin-fixed paraffin embedded (FFPE) tumours obtained at diagnosis. Hematoxylin-eosin-stained slides were analysed according to the current WHO 2007 criteria [20]. Large cell/anaplastic tumours (LCA) were diagnosed where anaplastic features were identified in the majority of analysed areas.

Additionally, genomic DNA extracted from the patient with *ALK* M1199L alteration and his parents' peripheral blood leukocytes was used to confirm the germline character and parental origin of identified alteration.

Informed consent was obtained to use tumour and blood material according to the procedures outlined by the CMHI's Ethical Committee.

Detection of molecular subtypes of tumours

The molecular subtypes of tumours were identified as follows:

1. WNT tumours by presence of at least two features as recommended by the International Medulloblastoma Working Group [12]: *CTNNB1* mutation, immunohistochemical positive nuclear reaction against β-catenin (BD #610154, San Jose, USA, dilution 1 : 800) and presence of chromosome 6 monosomy detected either by interface fluorescence in situ hybridisation (FISH) or by multiplex ligation-dependent probe amplification (MLPA).
2. SHH tumours by presence of immunohistochemical positive reaction with anti-GAB1 (Abcam, Cambridge, USA, #ab27439 and/or ab #59362, dilution 1 : 100) and anti-YAP1 (Santa Cruz Biotechnology, Dallas, USA, #sc-101199, dilution 1 : 50) antibodies, as described by Ellison *et al*. [9].
3. Non-WNT/SHH tumours were the remaining tumours, tested negative for the above features.

Mutations in exon 3 of the *CTNNB1*

Mutations in exon 3 of the *CTNNB1* gene were detected in genomic DNA obtained from frozen tumour tissues using the Sanger direct method. The

PCR reactions were carried out with the following primers: CTNNB1_3F:CCCTGGCTATCATTCTGCTT and CTNNB1_3R:TCTCTTTTCTTCACCACAACATTT using Amplitaq Gold DNA Polymerase (Roche, Basel, Switzerland) under the following conditions: 95°C for 8 min; 35 cycles of 95°C for 1 min; 57°C for 5 min; 72°C for 1 min, then a final extension step at 72°C for 7 min. Sequencing reactions were performed using a BigDye Terminator v.3.1 Cycle Sequencing Kit (Life Technologies) according to the manufacturer's protocol. Sequencing products were analyzed in ABI Prism 3130 Genetic Analyzer (Applied Biosystems, Foster City, CA, USA). Sequences of the analyzed fragments were compared with the *CTNNB1* cDNA (GenBank RefSeq: NM_001904.3) using Mutation Surveyor software version 3.30 (Soft Genetics, LLC, State Collage, PA, USA). The positions of the identified nucleotide changes were determined based on comparison with the reference sequence, with the A of the ATG translation initiation codon designated as nucleotide +1.

Multiplex ligation-dependent probe amplification

Multiplex ligation-dependent probe amplification was carried out on genomic DNA extracted from frozen tumour tissues for detection of copy number changes of chromosome 6. The analysis was performed using the SALSA MLPA kit P301-A2 (MRC-Holland, Amsterdam, the Netherlands) according to the manufacturer's protocol. Probe amplification products were run an ABI Prism 3130 Genetic Analyzer (Applied Biosystems, Foster City, CA, USA). Peak plots were visualized and normalized, and the dosage ratios were calculated using GeneMarker software v 2.2.0 (Soft Genetics, LLC, State Collage, PA, USA).

Fluorescence *in situ* hybridisation

Fluorescence in situ hybridisationwas performed on FFPE tissue preparations for detection of monosomy 6 using chromosome 6 Satellite Enumeration Probe (Kreatech, Amsterdam, the Netherlands) according to the protocols for the manufacturer of probes.

Detection of *ALK* variants and gene expression

A total of 20 ng of genomic DNA isolated from tumour tissue and/or peripheral blood lymphocytes by automated method (MagnaPure, Roche) was used to amplify coding and intronic flanking regions of exons 23 and 25 of the *ALK* gene.

The PCR reactions were carried out with the following primers: ALK_23F:GGAGCCTGCTGTGGTTCTTC and ALK_23R:AGTTGACACCCTGGGTTCC as well as ALK_25F:GGAAATATAGGGAAGGGAAGGAACTA and ALK_25R:TGATGTAAGGGACAAGCAGCC, using Amplitaq Gold DNA Polymerase (Roche, Basel, Switzerland) under the following conditions: 95°C for 8 min; 35 cycles at 95°C for 1 min; 62°C (exon 23)/60°C (exon 25) for 1 min; 72°C for 1 min, then a final extension step at 72°C for 7 min. Bidirectional sequencing was performed using a 3130 genetic analyser (Applied Biosystems, Foster City, CA, USA). The sequences were determined on both DNA strands from at least two independent PCR products. The analysed sequence fragments were compared with the ALK cDNA (GenBank RefSeq: NM_004304.4) and protein (GenBank RefSeq: NP_004295.2) sequences using Mutation Surveyor software version 3.30 (Soft Genetics, LLC, State Collage, PA, USA). Variant positions were numbered according to HGVS recommendations (with +1 corresponding to the A of the ATG translation initiation codon in the appropriate reference sequence).

Prediction of possible functional effects of novel alternations was performed using three different algorithms, which are web-based tools for the annotation of pathological variants in proteins: FATHMM (http://fathmm.biocompute.org.uk/index.html), PolyPhen-2 (http://genetics.bwh.harvard.edu/pph2/), and Mutation Taster (http://www.mutationtaster.org/). The identified novel variants were also screened against mutation and SNP databases: NCBI (www.ncbi.nlm.nih.gov/SNP) and the Human Gene Mutation Database Professional (HGMD; www.hgmd.cf.ac.uk/ac/validate.php). Additionally, the amino acid position of all selected changes in functional domains and posttranslational modifications was verified using NCBI Protein (http://www.ncbi.nlm.nih.gov/protein) and Alamut-2.4-6 Software.

Expression of ALK was detected by immunohistochemistry using antibody clone ALK1 (DAKO #M 7195, Agilent Technologies, Santa Clara, USA, dilution 1 : 20).

Results

Patients and tumours characteristics

Tumours from sixty four paediatric patients with medulloblastoma were analysed. The average age of patients at diagnosis was nine years, range 0.5-17 years. Forty patients were males, 24 patients were females.

Molecular groups included: ten WNT tumours, eight SHH tumours, 41 non-WNT/SHH tumours and 5 tumours without determined molecular features due to lack of tumour material for extensive analyses. A summary of patients and tumour characteristics is presented in Table I.

Detection of *ALK* variants

Among sixty four tumours, five variants were detected in exons 23 and 25 presented in Table II. In four cases they resulted in an intron variant without impact on the function of the encoded protein and were not considered further.

In one tumour, a novel heterozygous likely pathogenic variant c.3595A>T was detected in exon 23 that resulted in a methionine to leucine substitution at codon position 1199 (M1199L) in the kinase domain of ALK protein (Fig. 1A). Analysis of genomic DNA obtained from proband's peripheral blood leukocytes confirmed the germline nature of the

Table I. Characteristics of 64 patients with medulloblastoma analysed for *ALK* variants

	ALL	WNT	SHH	Non-WNT/SHH	Not determined
No. of patients	64	10	8	41	5
Age (years)					
Average; range	8.8; 0.5-17	9.3; 5-14	5.6; 0.5-11	9.2; 3-17	7.7; 2-15
0-3	5	0	4	0	1
4-17	59	10	4	41	4
Gender					
Male	40	5	3	30	2
Female	24	5	5	11	3
Histopathology					
Classic	44	10	1	32	1
LCA[1]	12	0	1	9	2
DN[2]	5	0	5	0	0
MBEN[3]	1	0	1	0	0
Not available	2	–	–	–	2
ALK status					
Variant	5	2	0	2	1
Normal	59	8	8	39	4

[1]LCA – large cell/anaplastic, [2]DN – desmoplastic/nodular, [3]MBEN – medulloblastoma tumour with extensive nodularity

Table II. Alterations in the *ALK* gene in five medulloblastoma tumours

Group	ALK alterations[1]		Function
	Exon 23	Exon 25	
WNT	c.3595A>T p. Met1199Leu	No change	Missense
WNT	No change	c.3744-32A>G (rs3738867)	Intron variant
Non-WNT/SHH	No change	c.3744-32A>G (rs3738867)	Intron variant
Non-WNT/SHH	No change	c.3744-32A>G (rs3738867)	Intron variant
Not determined	c.3516-62A>G (rs113866835)	No change	Intron variant

[1]Alterations include intronic flanking regions of exons 23 and 25

detected variant. Also maternal DNA sample showed c.3595A>T alteration indicating an inherited type of the alteration.

Variant M1199L is located in the inhibitor binding region of the kinase domain with no recorded SNP site. To predict whether the identified variant is tolerated or deleterious, we used the combined results of three different algorithms (see methods), which confirmed the pathogenicity of the novel p.Met1199Leu variant in the *ALK* gene (FATHMM, PolyPhen-2, Mutation Taster Scores: –2.51, 0.7; 0.99; respectively). A novel variant position relative to two hot spots mutations found in neuroblastoma and the only mutation previously detected in medulloblastoma is presented in Figure 1B.

Characteristics of the patient and tumour with c.3595A>T *ALK* likely pathogenic variant

A ten-year-old boy was diagnosed in 2008 with brain tumour in the posterior fossa midline location. The tumour was surgically totally removed and no metastases were detected on magnetic resonance imaging examination. The patient is still alive and has been disease free for 6 years since diagnosis.

Histopathologically the tumour was diagnosed as classic medulloblastoma, however with clearly visible areas of focal anaplastic features (Fig. 2A-C).

Analysis of DNA extracted from frozen tumour revealed presence of *CTNNB1* mutation in exon 3 (c.98C>A; p.S33Y) and loss of whole chromosome 6 detected by MLPA analysis. In addition, positive nuclear reaction against β-catenin was identified by immunohistochemistry (Fig. 2D) what altogether classifies this tumour to the WNT group. Presence of *ALK* expression at the protein level was confirmed by immunohistochemistry using ALK1 antibody (Fig. 2E).

Discussion

Up to now around 30 recurrent chromosomal translocations involving the *ALK* gene have been identified in different cancers, mainly anaplastic large cell lymphoma (ALCL), non-small cell lung cancer (NSCLC), inflammatory myofibroblastic tumour (IMT) and other tumours (review in [25]).

However, no fusion genes involving *ALK* have been found in neuroblastoma and recently described *ALK* translocations in neuroblastoma are not expected to result in a fusion transcript [10]. Therefore, mutations both somatic and germinal, leading to *ALK* activation are characteristic for this disease. It

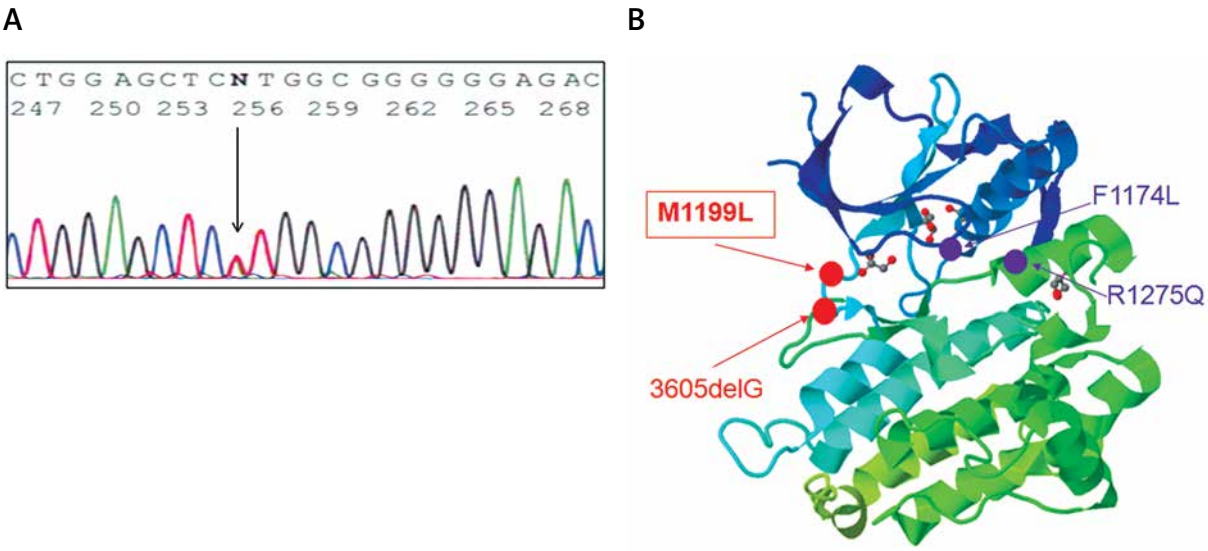

Fig. 1. *ALK* molecular variants in medulloblastoma and neuroblastoma. **A**) A novel *ALK* likely pathogenic variant c.3595A>T (M1199L) in exon 23 in medulloblastoma. **B**) A "ribbon" rainbow diagram of human ALK protein. Red dots indicate location of mutations in medulloblastoma. The M1199L variant detected in this study is located in a hinge region of the kinase domain of the ALK protein. Purple dots indicate two hot spots mutations in neuroblastoma. F1174 is located in C-helix region and R1275Q in the activation segment of the kinase domain of the ALK protein. The view is prepared from protein data bank file RCSB PDB ID: 3L9P.

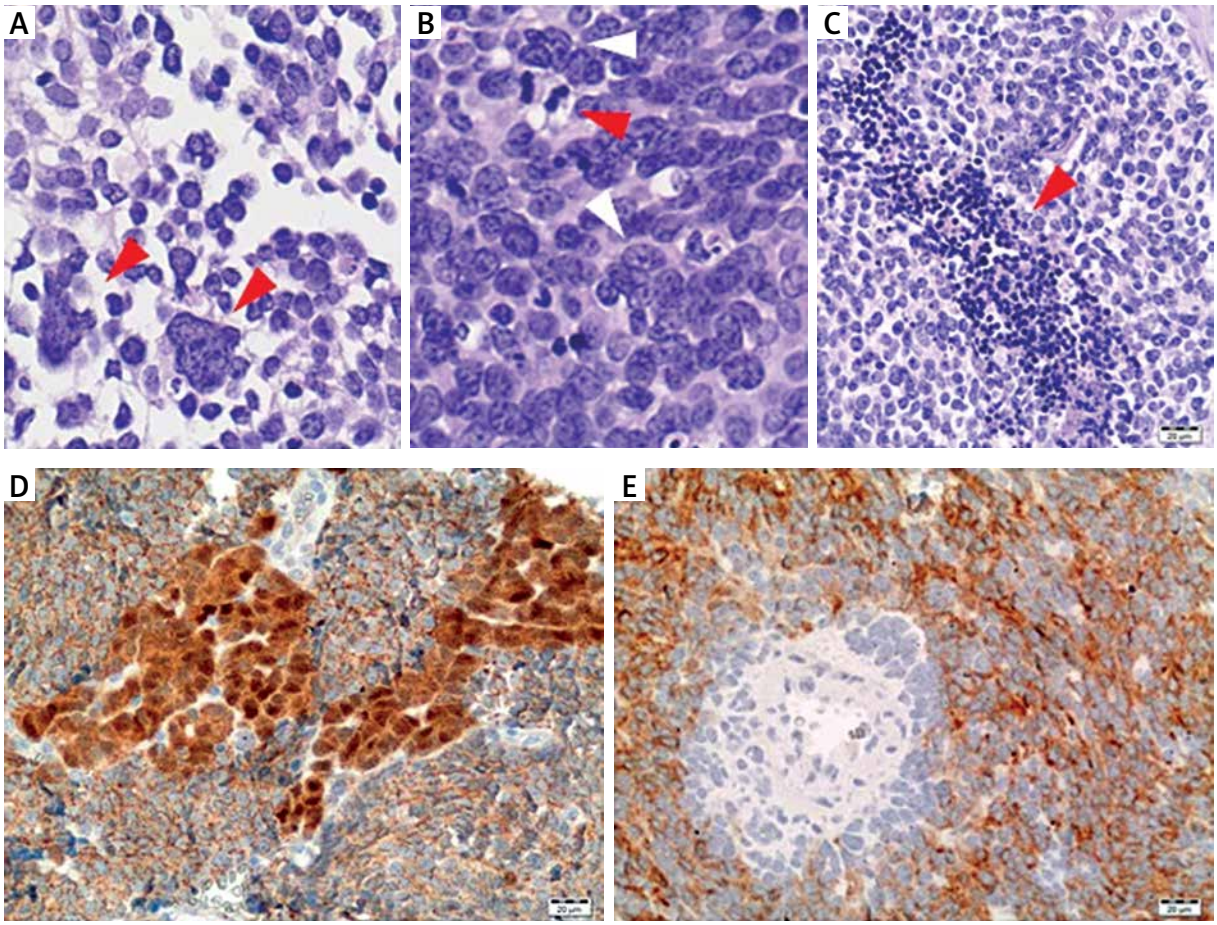

Fig. 2. Images of medulloblastoma tumour with presence of the M1199L variant. Hematoxylin-eosin-stained slides indicating presence of anaplastic features within the tumour (**A-C**). **A**) Red arrowheads indicate pleomorphic cells. **B**) Red arrowhead indicates mitotic cell and white arrowheads indicate cell-cell wrapping. **C**) Red arrowhead shows the area of apoptotic cells. **D**) Positive nuclear reaction against β-catenin. **E**) Positive reaction with anti-ALK1 antibody. Original magnification of images 200×.

is likely that additional genetic changes (e.g. *MYCN* amplification) are required for neuroblastoma tumorigenesis. Mouse model experiments revealed that knock-in mice carrying Alk F1178L mutation (equivalent to human F1174L) were characterized by an increased number of sympathetic neuroblasts and a prolonged proliferation of sympathetic neurons but they did not develop neuroblastoma tumours [2].

We have analysed 64 medulloblastoma tumours, including ten of the WNT type, and found a novel *ALK* M1199L likely pathogenic variant in a binding region of the kinase domain, likely to be deleterious and inherited from maternal origin. This variant may be linked to the ALK inhibitors resistance since the contact maintained by methionine residue M-1199 is most important for the effective binding and stability of the ALK-crizotinib complex [19]. The neighbouring region includes also the sites of secondary acquired *ALK* alterations L1996M and L1998P in EML4-ALK fusion positive lung cancers which confer resistance to crizotinib, and G1202R, which confers resistance to a second-generation ALK inhibitor alectinib and other ALK inhibitors [4,7,14,15]. Therefore, a precise character of the detected variant is critical for further therapeutic considerations.

Although the *ALK* gene was expressed at the protein level, it is possible that M1199L alteration is not sufficient for tumorigenesis alone and mutations in other genes are necessary for tumour development e.g. in *CTNNB1* or as yet unknown genes from chromosome 6. On the other hand, mouse experiments uncovered that activating mutations in *Ctnnb1* alone,

although caused the abnormal accumulation of cells on the embryonic dorsal brainstem, did not lead to the development of tumours [11]. This altogether emphasizes a requirement for a cooperative genetic event, similarly to neuroblastoma, in the development of the WNT type of medulloblastoma.

Germline deletion 3605delG found previously in medulloblastoma [5] is located within a short distance from the variant identified by us (c.3595A>T). However it is a nonsense mutation resulting in a frameshift producing a premature stop codon in exon 25 and putative truncated protein consisting of 1256 amino acids, as compared to 1620 of the wild-type. Its role in signal transduction remains to be determined. Also the molecular type of the tumour with *ALK* mutation was not established. Remarkably, both identified alterations occurred in tumours with presence of anaplastic features, which is in line with observation that *ALK* mutations are characteristic for de-differentiated tumours.

Very recent investigation of 37 medulloblastoma tumours did not reveal any mutations in exon 23 and exon 25 of the *ALK* gene [27]. It is likely that other alterations may be detected in medulloblastoma by application of e.g. next generation sequencing, similarly to intragenic *ALK* rearrangements detected recently in neuroblastoma [10].

In summary, this is the first evidence of inherited *ALK* likely pathogenic variant in the WNT type of medulloblastoma, what altogether with presence of ALK expression may point towards involvement of the *ALK* gene in this type of tumours.

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Disclosure

Authors report no conflict of interest.

References

1. Carén H, Abel F, Kogner P, Martinsson T. High incidence of DNA mutations and gene amplifications of the ALK gene in advanced sporadic neuroblastoma tumours. Biochem J 2008; 416: 153-159.
2. Cazes A, Lopez-Delisle L, Tsarovina K, Pierre-Eugène C, De Preter K, Peuchmaur M, Nicolas A, Provost C, Louis-Brennetot C, Daveau R, Kumps C, Cascone I, Schleiermacher G, Prignon A, Speleman F, Rohrer H, Delattre O, Janoueix-Lerosey I. Activated Alk triggers prolonged neurogenesis and Ret upregulation providing a therapeutic target in ALK-mutated neuroblastoma. Oncotarget 2014; 9: 2688-2702.
3. Cho YJ, Tsherniak A, Tamayo P, Santagata S, Ligon A, Greulich H, Berhoukim R, Amani V, Goumnerova L, Eberhart CG, Lau CC, Olson JM, Gilbertson RJ, Gajjar A, Delattre O, Kool M, Ligon K, Meyerson M, Mesirov JP, Pomeroy SL. Integrative genomic analysis of medulloblastoma identifies amolecular subgroup that drives poor clinical outcome. J Clin Oncol 2011; 29: 1424-1430.
4. Choi YL, Soda M, Yamashita Y, Ueno T, Takashima J, Nakajima T, Yatabe Y, Takeuchi K, Hamada T, Haruta H, Ishikawa Y, Kimura H, Mitsudomi T, Tanio Y, Mano H; ALK Lung Cancer Study Group. EML4-ALK mutations in lung cancer that confer resistance to ALK inhibitors. N Engl J Med 2010; 363: 1734-1739.
5. Coco S, De Mariano M, Valdora F, Servidei T, Ridola V, Andolfo I, Oberthuer A, Tonini GP, Longo L. Identification of ALK germline mutation (3606del) in pediatric anaplastic medulloblastoma. J Hum Genet 2012; 57: 682-684.
6. De Brouwer S, De Preter K, Kumps C, Zabrocki P, Porcu M, Westerhout EM, Lakeman A, Vandesompele J, Hoebeeck J, Van Maerken T, De Paepe A, Laureys G, Schulte JH, Schramm A, Van Den Broecke C, Vermeulen J, Van Roy N, Beiske K, Renard M, Noguera R, Delattre O, Janoueix-Lerosey I, Kogner P, Martinsson T, Nakagawara A, Ohira M, Caron H, Eggert A, Cools J, Versteeg R, Speleman F. Metaanalysis of neuroblastomas reveals a skewed ALK mutation spectrum in tumors with MYCN amplification. Clin Cancer Res 2010; 16: 4353-4362.
7. Doebele RC, Pilling AB, Aisner DL, Kutateladze TG, Le AT, Weickhardt AJ, Kondo KL, Linderman DJ, Heasley LE, Franklin WA, Varella-Garcia M, Camidge DR. Mechanisms of resistance to crizotinib in patients with ALK gene rearranged non-small cell lung cancer. Clin Cancer Res 2012; 18: 1472-1482.
8. Duijkers FA, Gaal J, Meijerink JP, Admiraal P, Pieters R, de Krijger RR, van Noesel MM. High anaplastic lymphoma kinase immunohistochemical staining in neuroblastoma and ganglioneuroblastoma is an independent predictor of poor outcome. Am J Pathol 2012; 180: 1223-1231.
9. Ellison DW, Dalton J, Kocak M, Nicholson SL, Fraga C, Neale G, Kenney AM, Brat DJ, Perry A, Yong WH, Taylor RE, Bailey S, Clifford SC, Gilbertson RJ. Medulloblastoma: clinicopathological correlates of SHH, WNT, and non-SHH/WNT molecular subgroups. Acta Neuropathol 2011; 121: 381-396.
10. Fransson S, Hansson M, Ruuth K, Djos A, Berbegall A, Javanmardi N, Abrahamsson J, Palmer RH, Noguera R, Hallberg B, Kogner P, Martinsson T. Intragenic anaplastic lymphoma kinase (ALK) rearrangements: Translocations as a novel mechanism of ALK activation in neuroblastoma tumours. Genes Chromosomes Cancer 2015; 54: 99-109.
11. Gibson P, Tong Y, Robinson G, Thompson MC, Currle DS, Eden C, Kranenburg TA, Hogg T, Poppleton H, Martin J, Finkelstein D, Pounds S, Weiss A, Patay Z, Scoggins M, Ogg R, Pei Y, Yang ZJ, Brun S, Lee Y, Zindy F, Lindsey JC, Taketo MM, Boop FA, Sanford RA, Gajjar A, Clifford SC, Roussel MF, McKinnon PJ, Gutmann DH,

Ellison DW, Wechsler-Reya R, Gilbertson RJ. Subtypes of medulloblastoma have distinct developmental origins. Nature 2010; 468: 1095-1099.
12. Gottardo NG, Hansford JR, McGlade JP, Alvaro F, Ashley DM, Bailey S, Baker DL, Bourdeaut F, Cho YJ, Clay M, Clifford SC, Cohn RJ, Cole CH, Dallas PB, Downie P, Doz F, Ellison DW, Endersby R, Fisher PG, Hassall T, Heath JA, Hii HL, Jones DT, Junckerstorff R, Kellie S, Kool M, Kotecha RS, Lichter P, Laughton SJ, Lee S, McCowage G, Northcott PA, Olson JM, Packer RJ, Pfister SM, Pietsch T, Pizer B, Pomeroy SL, Remke M, Robinson GW, Rutkowski S, Schoep T, Shelat AA, Stewart CF, Sullivan M, Taylor MD, Wainwright B, Walwyn T, Weiss WA, Williamson D, Gajjar A. Medulloblastoma Down Under 2013: a report from the third annual meeting of the International Medulloblastoma Working Group. Acta Neuropathol 2014; 127: 189-201.
13. Hallberg B, Palmer RH. Mechanistic insight into ALK receptor tyrosine kinase in human cancer biology. Nat Rev Cancer 2013; 13: 685-700.
14. Heuckmann JM, Hölzel M, Sos ML, Heynck S, Balke-Want H, Koker M, Peifer M, Weiss J, Lovly CM, Grütter C, Rauh D, Pao W, Thomas RK. ALK mutations conferring differential resistance to structurally diverse ALK inhibitors. Clin Cancer Res 2011; 17: 7394-7401.
15. Ignatius Ou SH, Azada M, Hsiang DJ, Herman JM, Kain TS, Siwak-Tapp C, Casey C, He J, Ali SM, Klempner SJ, Miller VA. Next-generation sequencing reveals a novel NSCLC ALK F1174V mutation and confirms ALK G1202R mutation confers high-level resistance to alectinib (CH5424802/RO5424802) in ALK-rearranged NSCLC patients who progressed on crizotinib. J Thorac Oncol 2014; 9: 549-553.
16. Janoueix-Lerosey I, Lequin D, Brugières L, Ribeiro A, de Pontual L, Combaret V, Raynal V, Puisieux A, Schleiermacher G, Pierron G, Valteau-Couanet D, Frebourg T, Michon J, Lyonnet S, Amiel J, Delattre O. Somatic and germline activating mutations of the ALK kinase receptor in neuroblastoma. Nature 2008; 455: 967-970.
17. Jones DT, Jäger N, Kool M, Zichner T, Hutter B, Sultan M, Cho YJ, Pugh TJ, Hovestadt V, Stütz AM, Rausch T, Warnatz HJ, Ryzhova M, Bender S, Sturm D, Pleier S, Cin H, Pfaff E, Sieber L, Wittmann A, Remke M, Witt H, Hutter S, Tzaridis T, Weischenfeldt J, Raeder B, Avci M, Amstislavskiy V, Zapatka M, Weber UD, Wang Q, Lasitschka B, Bartholomae CC, Schmidt M, von Kalle C, Ast V, Lawerenz C, Eils J, Kabbe R, Benes V, van Sluis P, Koster J, Volckmann R, Shih D, Betts MJ, Russell RB, Coco S, Tonini GP, Schüller U, Hans V, Graf N, Kim YJ, Monoranu C, Roggendorf W, Unterberg A, Herold-Mende C, Milde T, Kulozik AE, von Deimling A, Witt O, Maass E, Rössler J, Ebinger M, Schuhmann MU, Frühwald MC, Hasselblatt M, Jabado N, Rutkowski S, von Bueren AO, Williamson D, Clifford SC, McCabe MG, Collins VP, Wolf S, Wiemann S, Lehrach H, Brors B, Scheurlen W, Felsberg J, Reifenberger G, Northcott PA, Taylor MD, Meyerson M, Pomeroy SL, Yaspo ML, Korbel JO, Korshunov A, Eils R, Pfister SM, Lichter P. Dissecting the genomic complexity underlying medulloblastoma. Nature 2010; 488: 100-105.
18. Kool M, Koster J, Bunt J, Hasselt NE, Lakeman A, van Sluis P, Troost D, Meeteren NS, Caron HN, Cloos J, Mrsić A, Ylstra B, Grajkowska W, Hartmann W, Pietsch T, Ellison D, Clifford SC, Versteeg R. Integrated genomics identifies five medulloblastoma subtypes with distinct genetic profiles, pathway signatures and clinicopathological features. PLoS One 2008; 3: e3088.
19. Kumar A, Ramanathan K. Exploring the structural and functional impact of the ALK F1174L mutation using bioinformatics approach. J Mol Model 2014; 20: 2324.
20. Louis DN, Ohgaki H, Wiestler OD, Cavenee WK, Burger PC, Jouvet A, Scheithauer BW, Kleihues P. The 2007 WHO classification of tumours of the central nervous system. Acta Neuropathol 2007; 114: 97-109.
21. Mossé YP, Laudenslager M, Longo L, Cole KA, Wood A, Attiyeh EF, Laquaglia MJ, Sennett R, Lynch JE, Perri P, Laureys G, Speleman F, Kim C, Hou C, Hakonarson H, Torkamani A, Schork NJ, Brodeur GM, Tonini GP, Rappaport E, Devoto M, Maris JM. Identification of ALK as a major familial neuroblastoma predisposition gene. Nature 2008; 455: 930-935.
22. Northcott PA, Korshunov A, Witt H, Hielscher T, Eberhart CG, Mack S, Bouffet E, Clifford SC, Hawkins CE, French P, Rutka JT, Pfister S, Taylor MD. Medulloblastoma comprises four distinct molecular variants. J Clin Oncol 2011; 29: 1408-1414.
23. Pugh TJ, Weeraratne SD, Archer TC, Pomeranz Krummel DA, Auclair D, Bochicchio J, Carneiro MO, Carter SL, Cibulskis K, Erlich RL, Greulich H, Lawrence MS, Lennon NJ, McKenna A, Meldrim J, Ramos AH, Ross MG, Russ C, Shefler E, Sivachenko A, Sogoloff B, Stojanov P, Tamayo P, Mesirov JP, Amani V, Teider N, Sengupta S, Francois JP, Northcott PA, Taylor MD, Yu F, Crabtree GR, Kautzman AG, Gabriel SB, Getz G, Jäger N, Jones DT, Lichter P, Pfister SM, Roberts TM, Meyerson M, Pomeroy SL, Cho YJ. Medulloblastoma exome sequencing uncovers subtype-specific somatic mutations. Nature 2012; 488: 106-110.
24. Robinson G, Parker M, Kranenburg TA, Lu C, Chen X, Ding L, Phoenix TN, Hedlund E, Wei L, Zhu X, Chalhoub N, Baker SJ, Huether R, Kriwacki R, Curley N, Thiruvenkatam R, Wang J, Wu G, Rusch M, Hong X, Becksfort J, Gupta P, Ma J, Easton J, Vadodaria B, Onar-Thomas A, Lin T, Li S, Pounds S, Paugh S, Zhao D, Kawauchi D, Roussel MF, Finkelstein D, Ellison DW, Lau CC, Bouffet E, Hassall T, Gururangan S, Cohn R, Fulton RS, Fulton LL, Dooling DJ, Ochoa K, Gajjar A, Mardis ER, Wilson RK, Downing JR, Zhang J, Gilbertson RJ. Novel mutations target distinct subgroups of medulloblastoma. Nature 2012; 488: 43-48.
25. Roskoski R Jr. Anaplastic lymphoma kinase (ALK): Structure, oncogenic activation, and pharmacological inhibition. Pharmacol Res 2013; 68: 68-94.
26. Wang M, Zhou C, Sun Q, Cai R, Li Y, Wang D, Gong L. ALK amplification and protein expression predict inferior prognosis in neuroblastomas. Exp Mol Pathol 2013; 95: 124-130.
27. Yan B, Kuick CH, Lim M, Yong MH, Lee CK, Low SY, Low DC, Lim D, Soh SY, Chang KT. Characterization of anaplastic lymphoma kinase-positive medulloblastomas. J Clin Neurosci 2016; 23: 120-122.