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Loss of 10q26.1–q26.3 in association with 7q34–q36.3 gain or 17q24.3–q25.3 gain predict poor outcome in pediatric medulloblastoma

Annalisa Pezzolo [a,1], Simona Coco [b,1], Alessandro Raso [c], Federica Parodi [a], Angela Pistorio [d], Francesca Valdora [e], Valeria Capra [c], Massimo Zollo [f], Simona Aschero [g], Eleonora Basso [g], Armando Cama [c], Paolo Nozza [h], Claudio Gambini [h], Giuseppe Cinalli [i], Maria Luisa Garrè [c,j], Achille Iolascon [f], Vito Pistoia [a], Gian Paolo Tonini [b,*]

[a] Department of Experimental and Laboratory Medicine, IRCCS G. Gaslini Hospital, Genoa, Italy
[b] Translational Oncopathology, National Cancer Research Institute, Genoa, Italy
[c] Neurosurgery Unit, IRCCS G. Gaslini Hospital, Genoa, Italy
[d] Epidemiology and Biostatistics Unit, IRCCS G. Gaslini Hospital, Genoa, Italy
[e] Department of Oncology, Biology and Genetic (DOBiG), University of Genoa, Italy
[f] CEINGE, Advanced Biotechnologies, University of Naples, Italy
[g] Department of Pediatric Hematology/Oncology, University of Turin, Regina Margherita Children's Hospital, Turin, Italy
[h] Department of Pathology, IRCCS G. Gaslini Hospital, Genoa, Italy
[i] Pediatric Neurosurgery, Santobono-Pausilipon Children's Hospital, Naples, Italy
[j] Neuro-Oncology Unit, Department of Hematology-Oncology, IRCCS G. Gaslini Hospital, Genoa, Italy

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ABSTRACT

Medulloblastoma (MB) is the most common malignant brain tumor of childhood. We have investigated for novel chromosomal imbalances and prognostic markers of pediatric MB. Forty MBs out of 64, were analyzed using high resolution prometaphase comparative genomic hybridization. Chromosome 10q26.1–q26.3 loss combined with 17q24.3–q25.3 gain and/or 7q34–q36.3 gain in tumors predicted poor patient's survival. A minimal deleted region of 14.12 cM at 10q26.1–q26.3 was refined by LOH analysis. We propose a new prognostic marker for pediatric MB patient risk stratification based on the presence of 10q26.1–q26.3 loss plus 17q24.3–q25.3 gain and/or 7q34–q36.3 gain associations.

Abbreviations: MB, medulloblastoma; HRP-CGH, high resolution prometaphase comparative genomic hybridization; I-FISH, interphase fluorescent *in situ* hybridization; LOH, loss of heterozygosity; cnLOH, copy-neutral loss of heterozygosity; CNA, copy number aberration; WHO, World Health Organization; DAPI, 4′-6′-diamidino-2-phenylindole; FITC, fluorescein isothiocyanate; TRITC, tetramethyl rhodamine isothiocyanate; CCD, charge-coupled device; DMBT1, deleted in malignant brain Tumor 1 gene.

* Corresponding author. Tel.: +39 010 5737381.

E-mail addresses: annalisapezzolo@ospedale-gaslini.ge.it (A. Pezzolo), simona.coco@istge.it (S. Coco), rasoale@yahoo.it (A. Raso), federicaparodi@ospedale-gaslini.ge.it (F. Parodi), angelapistorio@ospedale-gaslini.ge.it (A. Pistorio), francesca.valdora@istge.it (F. Valdora), valeriacapra@ospedale-gaslini.ge.it (V. Capra), zollo@ceinge.unina.it (M. Zollo), simona.aschero@unito.it (S. Aschero), eleonora.basso@unito.it (E. Basso), armandocama@ospedale-gaslini.ge.it (A. Cama), paolonozza@ospedale-gaslini.ge.it (P. Nozza), claudiogambini@ospedale-gaslini.ge.it (C. Gambini), giuseppe.cinalli@gmail.com (G. Cinalli), mluisagarre@ospedale-gaslini.ge.it (M.L. Garrè), iolascon@ceinge.unina.it (A. Iolascon), vitopistoia@ospedale-gaslini.ge.it (V. Pistoia), gianpaolo.tonini@istge.it (G.P. Tonini).

[1] Contributed equally to this manuscript.

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1. Introduction

Medulloblastoma (MB) is a malignant, neuroepithelial embryonal tumor of the cerebellum with predominant neuronal differentiation and tendency to metastasize via cerebrospinal fluid pathways [1,2]. Staging systems for MB based on clinical parameters such as metastatic stage at diagnosis (M stage), patient's age and extension of surgery [3,4] are still common in practice but do not sufficiently reflect the tumors' heterogeneous nature. Although after eight decades since the first description of this neoplasm [5], the modern therapeutic regimens have significantly improved survival of affected children [6–8], the survival of these patients remains unsatisfactory.

The current World Health Organization (WHO) classification distinguishes MB into five histological variants: classic, nodular/desmoplastic, extensive nodularity, anaplastic and large-cell MB [1], however, the prognostic value of histological MB sub-types is still a matter of debate [9–12] although it is well known that anaplasia is associated with an aggressive course, whereas nodule formation with extensive neuronal differentiation predicts a favorable outcome [13,14].

Important signaling pathways such as *Wingless*, *Hedgehog* and *Notch*, which are involved in normal cerebellum development, are aberrantly activated in MB [15].

TRKC expression and β-catenin nuclear accumulation have been found suitable prognostic markers and are associated with a good prognosis, whereas *MYC* or *MYCN* gene amplification is associated with an adverse outcome [10,15].

Several chromosomal aberrations have been found in pediatric MBs, by cytogenetic analysis and comparative genomic hybridization (CGH) [16–27]. Isochromosome 17q, that is the most frequent aberration observed in about 40% of MB, is the result of chromosome 17p loss and gain of 17q [19,22,24,25]. The question raised from several observations is whether 17p loss or 17q gain (or both) are the critical events or whether the breakpoint itself is important in disrupting some crucial genes for MB development. Contradictory results have been reported about the role of 17p loss in this tumor [28,29].

Kool et al. [30] have classified MB in 5 subtypes based on gene expression profiling and they found chromosome 17p loss and 17q gain in two subtypes of MB associated with classic histology and metastatic disease. Moreover, Korshunov et al. [31] have demonstrated that DNA copy number variation likely contributes to pathogenesis of MB. Recently, we have performed a genome microarray analysis focused on classic MB and we have identified a significant association between 9q loss or 16q loss and unfavorable disease outcome [32].

The aim of the present study was to perform a comprehensive genome-wide analysis of pediatric MB including different histological types using a combined approach of high resolution prometaphase comparative genomic hybridization (HRP-CGH) and loss of heterozygosity (LOH), in order to identify new potential prognostic markers for this pediatric cancer.

2. Materials and methods

2.1. Tumor specimens and patients

Sixty-four tumor samples were collected from patients untreated at diagnosis by three research centers (IRCCS G. Gaslini Children's Hospital Genoa: 31 samples; CEINGE Advanced Biotechnologies, University of Naples: 14 samples, and Regina Margherita Children's Hospital University of Turin: 19 samples). The only inclusion criterion was the availability of the complete clinical records. Each tumor sample contained at least 85% malignant cells as assessed by histological analyses. Tumor histology was reviewed according to the latest WHO Classification [1]. Recurrent disease was diagnosed in the presence of metastatic disease. The clinical-pathological features of the patients and the molecular analyses carried out for each tumor are listed in the Supplementary Tables S1 and S2, respectively. Written informed consent was obtained from patients' parents or legal guardians, and local Ethics Committees for human studies from each Institution approved the research.

2.2. Nucleic acid extraction

Tumors were snap-frozen at the time of surgery and treated according to the recommendations of the European Society of Human Genetics [33]. Constitutional DNA was extracted from blood leukocytes of 51 out of 64 patients. Genomic DNA was isolated from frozen tumor samples and peripheral blood leucocytes by standard methods using a commercial kit (Invitrogen, Life Technologies, Carlsbad, CA, USA). DNA quantity and quality were determined by spectrophotometry (Biophotometer, Eppendorf AG, Hamburg, Germany) and agarose gel electrophoresis.

2.3. High resolution prometaphase comparative genomic hybridization (HRP-CGH)

HRP-CGH was performed using directly fluorochrome-conjugated DNA, as described previously [34]. Tumor and normal DNAs were labeled by nick translation using Spectrum Green-dUTP (2′-deoxyuridine 5′-triphosphate) and Spectrum Red-dUTP (Vysis Inc., Downers Grove, IL, USA), respectively. Labeled test and reference DNAs, together with Cot-1 DNA (Roche, Mannheim, Germany), were prepared as previously described [34]. Hybridization was performed on slides with prometaphase-like spreads leading to increase resolution of banding patterns for high resolution analysis. Ethidium Bromide (Sigma–Aldrich Co., St. Louis, MO, USA) was added to cultures prior to harvest to achieve longer chromosomes and improve resolution. Prometaphase-like chromosome preparations exhibit an average of 550–850 bands per haploid set. CGH preparations were analyzed with a Nikon Eclipse E1000 epifluorescence microscope (Nikon Corp., Tokyo, Japan) equipped with filter sets appropriate for DAPI (4′-6′-diamidino-2-phenylindole), FITC (fluorescein isothiocyanate), and TRITC (tetramethyl rhodamine isothiocyanate). Three single-color images corresponding to counterstain, test DNA, and