Chromosomal Translocations as a Mechanism of *BRAF* Activation in Two Cases of Large Congenital Melanocytic Nevi

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Genetic studies of melanocytic tumors have mainly demonstrated activation of oncogenes such as *NRAS* or *BRAF* through point mutations. In two cases of large congenital melanocytic nevi, we observed a chromosomal translocation involving the *BRAF* oncogene on chromosome 7q34, resulting in both cases in removal of the auto-inhibitory N-terminal regulatory domain (hence the Ras-guanosine triphosphate binding domain) of *BRAF* from its protein kinase domain. This is early evidence of *BRAF* activation through chromosomal translocation in melanocytic tumors. Because *BRAF* point mutations are rather rare in congenital melanocytic nevi and melanoma arising in non-sun-exposed area, the molecular mechanism of oncogenic activation as described here could be a recurrent molecular feature in these groups of melanocytic tumors.

Journal of Investigative Dermatology (2007) **127,** 1468–1470. doi:10.1038/sj.jid.5700725; published online 15 February 2007

INTRODUCTION

Although studies report an increased risk of melanoma in large congenital melanocytic nevi (LCMN), genetic investigations are rather rare in this type of melanocytic lesion (Berg and Lindelof, 2003; Ichii-Nakato *et al.*, 2006). Here we describe involvement of the *BRAF* oncogene through chromosomal translocation in two cases of LCMN.

RESULTS AND DISCUSSION

Cytogenetic analysis demonstrated a 46, XX, t(5;7)(q31;q34)[20] karyotype in case 1, and a 46, XY, t(2;7)(q24q33;q33q36)[20] karyotype in case 2 (Figure 1). Constitutional karyotypes were normal in both patients. Fluorescence *in situ* hybridization (FISH) analysis performed in both cases revealed splitting of the bacterial artificial chromosome RP11-25N5 (Figure 1), demonstrating the involvement of the *BRAF* gene in chromosomal translocation. Subsequent hybridizations with the bacterial artificial chromosome/P1 artificial chromosome – RP4-726N20 and RP5-839B19 – as well as with ''home-made'' probes (see Table S1) spanning the Ras-guanosine triphosphate binding domain (RBD) and protein kinase domain (PKD) of *BRAF* further delineated the breakpoint region between the PKD (remaining on the derivative chromosome 7) and the RBD (translocated to the derivative chromosome 5 in case 1 and to the derivative chromosome 2 in case 2) (data not shown). 5′ Rapid amplification of cDNA ends-PCR was performed in patient 1 using the 5′ rapid amplification of cDNA ends-PCR assay (Invitrogen, Carlsbad, CA), according to the manufacturer's instructions (see Table S1), and revealed a hybrid sequence including the 5′ part of the *FCHSD1* gene and the 3′ part of the *BRAF* gene, with a junction between *FCHSD1* exon 13 and *BRAF* exon 9. Reverse transcriptase-PCR confirmed the existence of the *FCHSD1-BRAF* fusion transcript using 5′ *FCHSD1* and 3′ *BRAF* primers (Figure 1), and showed the presence of the whole *BRAF* PKD included in its 3′ part (see Table S1 for primers used and Figure S1 for the fusion transcript cDNA and predicted amino-acid sequences). In case 2, no chimeric sequence could be identified either by 5′ rapid amplification of cDNA ends-PCR or by large genomic PCR. Western blots of lysates from cultured cells of patients 1 and 2 revealed increased phosphorylation of extracellular signal-regulated kinase 1/2 (p44/42 mitogen-activated protein kinase (MAPK)) compared with normal melanocytes (Figure 1), demonstrating activation of the MAPK pathway in these samples. In order to rule out the putative participation

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Abbreviations: CMN, congenital melanocytic nevi; FCH, FER/CIP4 homology; FISH, fluorescence in situ *hybridization; LCMN, large congenital melanocytic nevi; MAPK, mitogen-activated protein kinase; PKD, protein kinase domain; RAF, rapidly accelerated fibrosarcoma; RBD, Ras-guanosine triphosphate binding domain*

Received 20 September 2006; revised 3 November 2006; accepted 27 November 2006; published online 15 February 2007

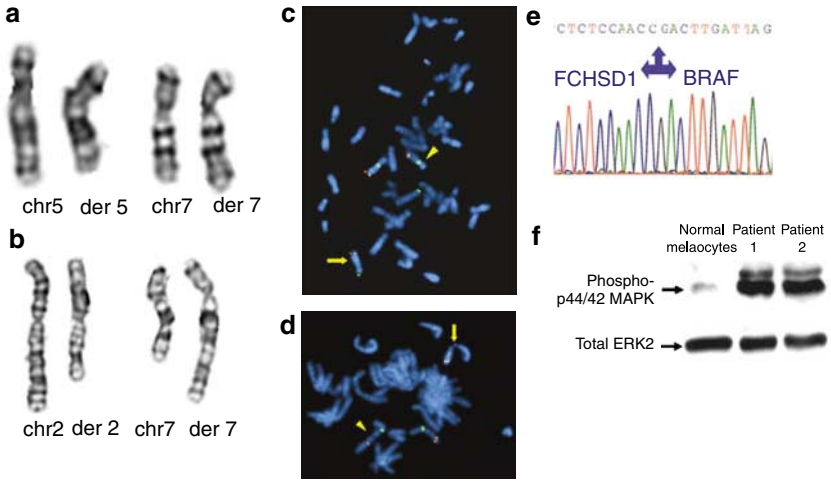

Figure 1. *BRAF* alterations in both cases studied. (**a**) Partial G-banding karyotype in patient 1 showing t(5;7)(q31;q34). (**b**) Partial G-banding karyotype in patient 2 showing t(2;7)(q24q33;q33q36). (**c**) Metaphase FISH of patient 1 showing splitting of the RP11-25N5 bacterial artificial chromosome (spectrum orange) with signals on both derivative (arrowhead) and normal chromosomes 7 and on the derivative chromosome 5 (arrow). Both normal and derivative chromosomes 7 and 5 were identified by Alpha CEP7 (spectrum green) and TelVysion 5p (spectrum green) probes, respectively. (**d**) Metaphase FISH in patient 2 showing splitting of the RP11-25N5 bacterial artificial chromosome (spectrum orange) with signals on both derivative (arrowhead) and normal chromosomes 7, and on the derivative chromosome 2 (arrow). Both normal and derivative chromosomes 7 and 2 were identified by Alpha CEP7 (spectrum green) and Alpha CEP2 (spectrum aqua) probes, respectively. (**e**) Sequence of the cDNA breakpoint junction in patient 1, showing partial *FCHSD1* and *BRAF* sequences in the 5′ and 3′ parts of the junction, respectively. (**f**) Western blots from normal melanocytes, and from tumoral melanocytes of patients 1 and 2, after 24 hours of serum depletion in the cell culture medium. A high basal activation of the MAPK pathway was observed in both patients 1 and 2, compared to normal melanocytes.

of *BRAF* and *RAS* point mutations in this MAPK pathway activation, sequencing of exons 11–17 of the *BRAF* gene, the whole *NRAS* coding sequence, and exons 1–4 of *KRAS* and 2–4 of *HRAS* genes (regions known to harbor all described mutations of these four genes in melanocytic tumors) was performed and failed to show any mutation (see Table S1).

We are thus dealing with two cases of LCMN presenting a novel mechanism of *BRAF* gene alteration. *BRAF* protein is one of the effectors belonging to the MAPK pathway and is composed of three functional domains conserved within the rapidly accelerated fibrosarcoma (RAF) kinase family: CR1 and CR2 in the N-terminal region, and CR3 in the C-terminal region. The CR1 domain includes the RBD, which mediates *RAS* binding, triggering the recruitment of *BRAF* protein to the cell membrane, and its kinase activation, whereas the CR3 domain contains the PKD (Mercer and Pritchard, 2003). In patient 1, t(5;7)(q31;q34) translocation resulted in the fusion of the 5′ part of the *FCHSD1* gene to the 3′ part of the *BRAF* gene on derivative chromosome 7. Although the exact function of the *FCHSD1* gene is still undetermined, the protein is known to be characterized by an FER/CIP4 homology (FCH) domain located in its N-terminal portion and two SH3 domains in its C-terminal portion (Katoh and Katoh, 2004). FCH domains are involved in the regulation of cytoskeletal rearrangements (Aspenström, 1997), whereas SH3 domains are described as protein-interaction modules playing critical roles in a wide variety of biological processes by binding to proline-rich ligands (Mayer, 2001). The hybrid protein generated by the chimeric gene thus contains the FCH domain of *FCHSD1* in its N-terminal part, and the PKD domain of *BRAF* in its C-terminal portion, with loss of the auto-inhibitory RBD domain.

In patient 2, the chimeric gene generated by the t(2;7)(q24q33;q33q36) translocation could not be determined despite several PCR attempts, but FISH results allowed us to assume that the same molecular alteration of the *BRAF* gene was present in this case, hence the removal of the auto-inhibitory RBD domain from its PKD domain.

It has been demonstrated that removal of the N-terminal regulatory domain of the *RAF* gene results in a kinase domain with high basal activity independent of *RAS* activation (Chong and Guan, 2003). Although uncommon, an oncogenic fusion transcript involving *BRAF* has also been reported in thyroid papillary carcinoma (Ciampi *et al.*, 2005). This fusion transcript resulted from a paracentric inversion of chromosome 7 and was composed of the 5′ part of the *AKAP9* gene (exons 1–8) and the 3′ part of *BRAF* (exons 9–18), including its PKD domain. Interestingly, the hybrid protein, for which increased kinase activity as well as transforming properties were demonstrated, had lost the *BRAF* auto-inhibitory N-terminal portion, as observed in our two cases. Thus, the release of the *BRAF* PKD domain from its auto-inhibitory domain seems to be the common point between our two cases and those reported by Ciampi *et al.* (2005). The gene partners are different in each situation and probably have no fundamental role in the *BRAF* activation generated by the translocation. The role of the partner gene may be limited to providing an efficient promoter driving the expression of constitutively activated *BRAF*.

In the present cases, the mitotic events generating the translocations would have to have occurred relatively early in embryogenesis to produce such LCMN. These events would arise from error-prone non-homologous end-joining repair of

double-strand breaks in DNA (Xiao *et al.*, 2001). Translocations of chromosome 7 with either chromosome 5 or chromosome 2 in our two patients may be not fortuitous but, rather, related to their proximity to interphase melanocytic nuclei, as described for other tumor-associated chromosomal translocations (Neves *et al.*, 1999).

The apparently rare occurrence of chromosomal translocations involving *BRAF* in melanocytic tumors could be the result of the small number of reported studies dealing with karyotype analyses in this type of neoplasia. On the other hand, point mutations (especially the *BRAF*V600E mutation) are the main molecular mechanism of *BRAF* activation in both benign and malignant acquired melanocytic tumors (Davies *et al.*, 2002; Pollock *et al.*, 2003). However, *BRAF* mutations are rarer in medium CMN and LCMN (De Raeve *et al.*, 2006; Ichii-Nakato *et al.*, 2006). It may be that translocations involving *BRAF* represent an alternative mechanism of its activation in CMN that harbor neither a *BRAF* nor an *NRAS* mutation and in melanoma from chronically sun-exposed or non-sun-exposed skin (for which BRAF mutations are also rare, as noted in medium CMN and LCMN). Other karyotypic studies on CMN and other melanomas are needed to corroborate this hypothesis.

MATERIALS AND METHODS

Primary melanocytic cell cultures were performed from tumoral samples as described previously (Morandini *et al.*, 1998). Informed consent was obtained from the patients, and the study was approved by the Ethical Committee of the Faculty of Medicine (Université Libre de Bruxelles). The study was conducted according to the Declaration of Helsinki Principles. For both cases, G-banding karyotypes were expressed according to the International System for Human Cytogenetic Nomenclature (1995). FISH was performed with BAC/PAC (supplied by Archives Group at the Sanger Institute, Cambridge, UK) labelled using the Vysis Nick Translation Kit (Downers Grove, IL). The different PCR primers as well as PCR protocols used are detailed in Table S1. Western blotting analyses were performed on proteins lysates from normal and tumoral melanocytes after 24 h of serum depletion. These experiments were reproduced on two independent lysates for each patient and performed with anti-phospho-p44/42 MAPK (Cell Signaling Technology, Beverly, MA) or anti-total ERK2 (extracellular signal-regulated kinase 2) antibodies (Santa Cruz Biotechnology, Santa Cruz, CA).

CONFLICT OF INTEREST

The authors state no conflict of interest.

ACKNOWLEDGMENTS

This work was supported by the F.N.R.S. (Grant F.N.R.S. – Télévie) and by the CHC (Children Hope Challenge). We thank Isabelle Borsu, Marie-Jeanne Gebski, Chantal Debussher, Bruno Pichon, Sandra Strollo, Xavier Pesesse, and Laurent Verbruggen for their excellent technical assistance.

SUPPLEMENTARY MATERIAL

Table S1. List of forward and reverse primers and respective PCR conditions used to make FISH probes, to perform 5′ rapid amplification of cDNA ends-PCR and reverse transcriptase-PCR investigations, and for *BRAF*, *NRAS*, *HRAS*, and *KRAS* genes sequencing.

Figure S1. *FCHSD1-BRAF* fusion cDNA sequence and predicted amino-acid sequence for patient 1.

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