ID: 00717
Type: COMUNICACIÓN ORAL
Topic: 4. Research and diagnostic platforms

SETD6 dominant negative mutation in Familial Colorectal Cancer Type X

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Introduction

Familiar colorectal cancer type X (FCCTX) comprises families fulfilling the Amsterdam criteria for Hereditary Non-Polyposis Colorectal Cancer, but that lack the mismatch repair deficiency that defines Lynch syndrome. Thus, the genetic cause that increases the predisposition to colorectal and other related cancers in families with FCCTX remains to be elucidated. Using whole-exome sequencing, we identified a truncating mutation in the *SETD6* gene in all the affected members of a FCCTX family. SETD6 is a mono-methyltransferase known to regulate by methylation RelA and PAK4, from the NF-?B and the Wnt pathways, respectively. In addition, recent studies have also been linked SETD6 to the regulation of the nuclear hormone receptor signaling, embryonic stem cell differentiation and oxidative stress response. Given that many of these pathways have been reported to be involved in cancer initiation and progression, this variant could potentially explain the increased cancer risk in this family.

Objective

The aim of this study was to functionally characterize this *SETD6* mutation in order to clarify its implication in the cancer inheritance of this FCCTX family. The working hypothesis was that the variant would affect the normal function of the enzyme, resulting in an alteration of the different pathways in which it is involved.

Methods

The whole exome of three members of a FCCTX family was obtained by Next Generation Sequencing (NGS). The segregation and loss of heterozygosity (LOH) were studied for all the candidate variants identified, among which a mutation in *SETD6* was selected for further characterization. For this purpose, the truncated version of the enzyme was cloned, expressed and purified. The recombinant protein was then used to test the activity and binding properties of the mutant enzyme in a hot cell-free *in vitro* methylation assay and an ELISA, respectively. In addition, the truncated SETD6 was overexpressed in the colon cancer cell line HCT116, followed by chromatin immunoprecipitation, so as to check the localization, substrate-binding ability and methylation capability in human cells. Finally, the allele-specific expression was analyzed in the tumor of one of the carriers and a competition assay was carried out.

Results and conclusion

After thorough filtering of the variants detected in a FCCTX family, a rare truncating mutation in the *SETD6* gene (c.791_792insA, p.Met264IlefsTer3) was selected for additional research. This variant has a minor allele frequency of 0.001, and it shows a complete segregation within the family, while no LOH is observed in the tumor. Moreover, this is a frameshift variant and, although the resulting mutant SETD6 retains its catalytic domain, it loses the C-terminal half of the protein. Here we demonstrate that, although the truncated SETD6 displays similar localization and substrate-binding ability as the wild-type protein, it lacks its enzymatic activity as a methyltransferase. In

addition, we show that both the wild-type and mutant alleles are expressed in the tumor of one of the carriers and that the two forms of SETD6 compete for their substrates, pointing to a dominant negative role.

Together, our findings suggest that SETD6 c.791_792insA (p.M264Ifs*3) is a dominant negative truncating mutation that impairs the normal function of SETD6, what may result in the deregulation of the different pathways in which it is involved and contribute to the increased susceptibility to cancer in this FCCTX family. These results certainly point to a pathogenic role of this mutation, though not enough to prove that SETD6 alone is responsible for their increased cancer risk, since other factors might be also involved. While the current work focuses on SETD6, we cannot exclude the possibility that the additional candidate variants identified may contribute independently or together to the pathology of FCCTX. Although no other SETD6 variants were found in the remaining families that were studied, the screening of this gene in a larger group of patients could provide more insights into its role in other FCCTX families.