Genetic analysis of *SMAD4* C-terminal domain in patients with microsatellite stable early-age onset colorectal cancer

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Background/Introduction: SMAD4 protein loss is a relatively common feature of sporadic colorectal cancers (CRC), and it was observed to be even more frequent in early-age onset CRC patients and microsatellite stable (MSS) tumors. Pathogenic variants in the *SMAD4* gene are usually missense or nonsense mutations, and they are more frequent in the C-terminal domain. The aim of this study was to perform genetic analysis of *SMAD4* C-terminal domain of MSS early-age onset CRC patients. This pilot study was conducted with a purpose of investigating if such genetic screening strategy would be useful for diagnostic purposes in this specific subgroup of CRC patients.

Method: This study included 20 patients with MSS early-age onset CRC (less than 50 years at the time of diagnosis). Tumoral DNA was extracted, and genetic analysis of *SMAD4* C-terminal coding exons 9, 10, 11 and 12 was performed by PCR and direct DNA sequencing.

Results: Among the 20 analyzed tumor DNAs, one sample was found to harbor a *SMAD4* variant: NC_000018.9:g.48591918C>T; (NM_005359.5: c.1081C>T; Arg361Cys). The variant was discovered in exon 9, affecting the codon 361, which represents a mutational hot spot within the *SMAD4* gene. This variant was discovered in homozygous state in the tumor of a 47-years old female with T3 stage carcinoma of the right colon. The variant has been functionally characterized before as pathogenic.

Conclusion: A *SMAD4* c.1081C>T pathogenic variant was discovered in the exon 9 known as a mutational hot spot in one individual with MSS early-age onset CRC. Considering the incidence and functional consequences of *SMAD4* exon 9 variants, the screening of this region could be a useful low cost strategy for the genetic analysis of colorectal tumors from patients with early-age onset, as well as for susceptibility testing.