Searching for new candidates genes in patients suspected of Lynch syndrome

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Lynch syndrome (LS), otherwise known as Hereditary Non Polyposis Colorectal cancer (HNPCC), is an autosomal dominant genetic disorder related to a high risk of colon cancer as well as other cancers, including endometrium and ovary. LS is defined by germline inactivation in one of the DNA mismatch repair (MMR) genes, mostly in *MLH1* and *MSH2,* but also *MSH6, PMS2* and *PMS1.* Yet, a significant number of cases highly suspected of LS remain without a genetic explanation suggesting that other genes could be potentially responsible for Lynch-like syndrome. Our previous study in 116 Brazilian patients suspected of LS, available at the AC Camargo Cancer Center, Sao Paulo, revealed 39% MMR mutation carriers (Silva et al., 2015), consequently, remaining 61% of patients could be subjects for searching pathogenic mutations in novel genes. Our current goal is to characterize new candidate genes involved in Lynch syndrome based on this previously characterized patient cohort.

Material and Methods: Whole exome sequencing (WES) on the SOLiD 5500 platform was carried out in blood samples of five members (three affected and two non-affected) from one LS suspected family that fulfilled the Amsterdam criteria and showed no mutation in the five MMR genes. Additional RNA samples, available later from the affected members of this family, included one tumor sample (proband) and two tumor-adjacent normal tissue samples (proband and her aunt). RT-PCR and pyrosequencing were used for screening splice variants and assessing allelic imbalance, respectively. Independent controls not carrying mutations of our interest included three colorectal tumors of other origin and five blood samples from normal volunteers.

Results: WES analysis of genetic variants segregating with LS revealed only two novel candidates, namely a single nucleotide deletion in *WDR27* (NM_001202550.1:c.1844delG) gene and a silent mutation in the *KIT* gene (NM_000222.2:c.1983C>A, p.Thr661Thr) in the end of exon 13, 7 nt upstream the splice site. These genetic variants were not reported in population based SNP databases and in ~250 healthy Brazilian controls of our previous study. Questioning the possible effect of this KIT mutation on splicing, we used bioinformatics prediction through Human Splicing Finder that suggested two sites broken for SR splicing factors. None splicing variants were identified in tumor or blood samples from the *KIT* c.1983C>A carriers until now. However, we have showed a strong *KIT* allelic expression imbalance (the mutated allele 3.6-fold more expressed) in the proband colorectal tumor while the alleles were equally expressed in both tumor-adjacent normal tissue samples available.

Perspectives: We currently continue our efforts to uncover the mechanisms of affecting the *KIT* expression by the novel mutation and to better characterize its pathogenic potential. Other strategies for splicing screening including minigene tests will be carried out. Identification of novel genes associated to Lynch-like syndrome is of crucial importance for genetic characterization of this not so well understood disorder and has direct consequences for the patients diagnosed as suspected of LS with genetic cause unknown.

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