Mutational status of main oncogenes and microsatellite instability in Sardinian patients with advanced colorectal cancer
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Colorectal cancer (CRC) is the third and second most common malignancy in men and women, respectively. Approximately, one in four CRC patients is diagnosed with an advanced disease (stage IV, according to the American Joint Committee on Cancer/AJCC). Advanced CRC has a high mortality rate, consistently ranking in the top three causes of cancer-related deaths, whereas treatment success rates range from 70-90% in the localized disease. CRC is predominantly associated with developed countries and a Western lifestyle and diet.

In CRC pathogenesis, somatic mutations in \textit{RAS} and \textit{BRAF} oncogenes play an important role and represent a key focus for the use of targeted therapies. In recent past years, the epidermal growth factor receptor (EGFR)-depending pathway has been largely exploited for personalized therapies and EGFR has become a key target of specific inhibitors to treat metastatic CRCs. Activating mutations in \textit{RAS} are recognized as a strong predictor of resistance to EGFR-targeted agents, since they cause a constitutive phosphorylation of the RAS proteins - independent on activation status of the upstream EGFR protein - which in turn permanently promote cell proliferation and drastically reduce the effects of the EGFR inhibition. In particular, monoclonal antibodies targeting EGFR or the vascular endothelial growth factor (VEGF) are biologic-targeted agents, both of which have improved outcomes when utilized in combination with chemotherapy in metastatic CRC. In particular, EGFR inhibitors are included into the treatment of CRC with \textit{RAS} and \textit{BRAF} wild-type status, the anti-VEGF agent bevacizumab in \textit{RAS}-mutated CRCs, and \textit{BRAF} inhibitors in \textit{BRAF}-mutant tumors. Moreover, occurrence of microsatellite instability (the MSI phenotype), which is present in <15% of CRCs, predicts positive response to immune checkpoint inhibitors (ICI).

In past years, we evaluated the contribution of \textit{RAS} and \textit{BRAF} mutations in a large collection of CRC patients from Sardinia (N=2,376). We are actually investigating the impact and relationship of the KRAS, NRAS, and BRAF mutational status with the MSI phenotype in a recent subset of Sardinian patients with metastatic CRC. A total of 105 patients with histologically-proven diagnosis of metastatic CRC at the time of enrolment, who were treated and followed-up at healthcare institutions across Sardinia, were included into the study. Genomic DNA was isolated from formalin-fixed, paraffin-embedded primary tumor tissue samples from CRC patients and screened for mutations in \textit{RAS} and \textit{BRAF} genes, using pyrosequencing or real-time PCR (Idylla) assays.

The MSI status was determined by comparison of genomic DNAs from tumor and normal tissues using a panel with five marker loci consisting of two mononucleotide repeats (BAT26, BAT25) and three dinucleotide repeats (D2S123, D5S346, and D17S250). To date, mutation rates of KRAS, NRAS, and BRAF were 44.8% (47/105), 5.7% (6/105), and 3.8% (4/105), respectively. A high-MSI (H-MSI) prevalence was found in 9.5% (10/105) cases, roughly in agreement with literature data; among them, 50% (5/10) were RAS mutated, 1% (1/10) BRAF mutated and 3% (3/10) RAS-BRAF wild type. We intend to increase the series and collect details about the clinical and pathological information of all patients included into the study in order to perform correlation evaluations. Providing further clues about the predictive and prognostic roles of molecular data in this setting of patients will enable the clinical management and decisions on individualized therapy in future.

Keywords: Colorectal cancer, RAS-BRAF genes, microsatellite instability.