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Liquid biopsy in metastatic colorectal cancer patients: determination of RAS mutational status and analysis of circulating exosomes

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Introduction

Liquid biopsy offers a non-invasive method for detecting specific cancer biomarkers in blood and it is emerging as one of the most promising tools in clinical oncology. The term liquid biopsy involves the analysis of several components such as circulating tumor DNA (ctDNA), circulating tumor cells, exosomes and circulating microRNAs (miRNA), although new strategies are day-to-day appearing to increase the possibility of detecting new biomarkers. Anti-EGFR monoclonal antibodies should only be considered for treatment of metastatic colorectal cancer (mCRC) patients whose tumors are wild type for RAS genes. FFPE (formalin-fixed paraffin-embedded) tumor tissue is currently used as Standard of Care for RAS testing, however, it is an invasive process and could involve some risks for patients. In patients with cancer, a fraction of cfDNA is tumor derived and is termed circulating tumor DNA (ctDNA). Because tumor-specific alterations in ctDNA are not present in normal cells, they offer an exquisitely sensitive and specific approach for cancer detection. The assay of ctDNA allows the analysis of tumors that are in areas of difficult access and lesions with insufficient material available for genotyping. Also, tumor heterogeneity, both intratumoral and between primary lesions and metastases, could be approached through ctDNA analysis. On the other hand, exosomes are implicated in intercellular signaling and as regulators of tumor progression. Exosomes have been shown to carry different proteins and nucleic acids, including miRNAs. The analysis of protein and miRNA profiles in circulating exosomes, may therefore help to examine whether RAS status affects exosome composition and behavior, and could have a prognostic and/or predictive value for the response to anti-EGFR treatment.

Objectives

The two main objectives of this study were to determine the level of concordance between plasma and tissue RAS mutation status in patients with mCRC and to establish a differential profile of the composition of plasma exosomes between RAS mutated and RAS wild type mCRC patients.

Methods

RAS testing was performed on 87 plasma samples using BEAMing, and compared with data of corresponding FFPE tumor samples. Then, for the digital PCR an emulsion was made with magnetic beads. Emulsion was broken and a hybridization PCR was performed with fluorescent probes. Finally, a flow citometry was carried out to analyse wild type and mutated DNA. When RAS mutational status was established, exosomes were isolated from the same blood sample obtained for BEAMing. CaptEV and ExoIP Composite Kits allowed the inmunocapture of these extracellular vesicles. Purified exosomes were observed by electron microscopy and some of the most

abundant exosome marker proteins in these vesicles were analysed. Two groups of patients were established depending on RAS mutational status and, the levels of expression of 752 miRNAs were analysed in each patient using OpenArray technology.

Conclusion

The overall agreement (concordance) of RAS mutations detected in plasma and in tumor tissue was 87,4%. The positive agreement was 89,29% (50/56), and the negative agreement was 83,87% (26/31). The prevalence of RAS mutations was 64,37% (56/87) and 63,22% (55/87) detected in plasma and tumor tissue, respectively. All patients with mutation detected in both tissue and blood had a 100% of concordance between the mutated codons. The vast majority of mutations detected were KRAS codons 12 and 13. The high concordance between plasma and tissue results demonstrates that BEAMing is a blood-based RAS mutation test with comparable capacity to detect RAS mutation astissue-based RAS determination. Therefore, testing of RAS mutational status in plasma is a powerful tool for the diagnosis and clinical management of mCRC patients that could replace tumor tissue analysis. We also quantified total cfDNA in 133 mCRC patients and found that there were significant higher cfDNA levels in RAS mutated patients. In addition, there was a significant correlation between cfDNA and the levels of one of the most commonly used CRC biomarker, CEA (carcinoembryogenic antigen), whereas there was no significant correlation between CEA and another biomarker such as CA 19-9. This result indicates a potential clinical utility of cfDNA as a biomarker in colorectal cancer. There was also a significant correlation between cfDNA levels and the mutant RAS allelic fraction. Regarding exosomes, we confirmed their purification from plasma by electron microscopy and by analysing specific exosomal markers, such as HSP70, Annexin V, CD9, Alix and Flotilin-1. Distinct exosomal protein and miRNA profiles were found depending on RAS mutational status. The analysis of circulating exosomes composition in mCRC patients may therefore help in the development of novel prognostic and/or predictive biomarkers for the response to anti-EGFR treatment.