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Dicer and Drosha expression and response to Bevacizumab-based therapy in advanced colorectal cancer patients

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Abstract ***Purpose:*** The miRNA-regulating enzymes Dicer and Drosha exhibit aberrant expression in several cancer types. Dicer and Drosha play a crucial role during the angiogenetic process in vitro and, for Dicer, in vivo. We aimed to investigate the potential role of Dicer and Drosha in predicting response to Bevacizumab-based therapy in advanced colorectal cancer (CRC) patients.

Methods: Dicer and Drosha mRNA levels were analysed in formalin-fixed paraffin-embedded specimens from patients affected by advanced CRC treated with or without Bevacizumab-containing regimens ($n = 116$ and $n = 50$, respectively) and from patients with diverticulosis as control group ($n = 20$). The experimental data were obtained using qRT-PCR, analysed comparing Dicer and Drosha expression levels in tumour samples versus normal mucosa and then compared to clinical outcome.

Results: The tumour samples from Bevacizumab-treated patients showed a significantly higher Drosha expression ($P < .001$) versus normal mucosa, while Dicer levels did not differ. Intriguingly, we found that low Dicer levels predicted a longer progression-free survival (PFS)

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($P < .0001$) and overall survival (OS) ($P = .009$). In addition, low Dicer levels were associated with better response to Bevacizumab-based treatments versus high Dicer levels (1.7% complete responses and 53.4% partial responses versus 0% and 32.7%, respectively; $P = .0067$). Multivariate analysis identified three independent predictors of improved OS: high performance status (PS) (relative risk (RR) 1.45; $P = .011$), lower organs involvement (RR 0.79; $P = .034$) and low Dicer expression (RR 0.71; $P = .008$). Conversely, Drosha levels were not associated with prognosis and outcome associated with treatment. In non-Bevacizumab-treated patients, Dicer and Drosha expression did not correlate with outcome.

Conclusion: These findings suggest that low Dicer mRNA levels seem to be independent predictors of favourable outcome and response in patients affected by advanced CRCs treated with Bevacizumab-based therapy.

1. Introduction

Colorectal cancer (CRC) is the third world's leading cause of cancer death. About 40–50% of newly diagnosed patients account for a metastatic disease, which is associated with high mortality.[1] The pathogenesis of CRC is a complex process, tightly controlled by multiple regulatory mechanisms including genome structure rearrangements, chromatin remodelling, epigenetic alterations and genetic mutations.[2] In the past few years, a gradually increasing number of studies documented that these processes are regulated by a class of small noncoding RNAs called microRNAs (miRNA) and involved in a wide spectrum of biological processes.[3]

Recent evidences have shown that alteration in miRNA expression is involved in the pathogenesis of cancers and in the metastatization process. The master regulators of miRNA biogenesis are two ribonucleases called Dicer and Drosha that act at different stages of miRNA synthesis and maturation. In the "miRNA machinery", Drosha is involved in the initial step of miRNA processing in the nucleus, where short (60–70 nucleotides) double-stranded RNA precursors (pre-miRNAs) are generated.[4]

Subsequently, the resulting pre-miRNA is exported to the cytoplasm and then cleaved by Dicer to generate the mature products, double-stranded miRNA fragments of 15–30 nucleotides.[5–7]

Some studies suggest that these factors, required for the biogenesis of miRNAs, are also implicated in cancer development. Growing evidences indeed show that Dicer and Drosha expression levels may vary among tumour types, but the regulation of these genes is still unclear. Recently, Karube et al. indicated that levels of Dicer could be used as prognostic markers in non-small cell lung cancer (NSCLC) and in breast cancer patients, showing that reduced messenger RNA (mRNA) expression is significantly associated with poor patient survival.[8,9] Moreover, Merritt et al. demonstrated that levels of Dicer and Drosha are prognostic factors in patients with ovarian cancer.[10]

In CRC, it has been demonstrated that a high expression (both at mRNA and protein level) of Dicer is significantly related to poor survival, independent of gender, age, tumour site, stage and differentiation.[11,12]

Intriguingly, several studies have shown that Dicer and Drosha play a crucial role during the angiogenic process in vitro and that Dicer is also involved in the angiogenesis regulation in in vivo models.[13] In fact, genetic silencing of Dicer in a mouse model was found to impair normal morphogenesis and organ development due to a de-regulation of angiogenesis-related genes.[14]

Nowadays Bevacizumab, a humanised recombinant monoclonal antibody that inhibits vascular endothelial growth factor A (VEGF-A), is part of the standard first-line treatment for metastatic CRC.[15,16]

Based on these data, our aim was to investigate the expression of Dicer and Drosha and their role as prognostic and predictive factors of response to Bevacizumab-based treatment in advanced CRC patients.

2. Patients and methods

2.1. Exploratory review of microarray data

We decided to query the cancer microarray database Oncomine™ (Compendia Bioscience, Ann Arbor, MI, USA, version 4.4) for the mRNA expression of Dicer and Drosha, in order to have a large overview of the expression of our genes of interest across existing datasets. We decided to set a threshold *P*-value of 0.05 and fold change of 2 in order to include comparisons in our exploratory analysis. A gene/probe had to appear in the top 10% of the ranking to include the series in the analysis. The co-expression analysis in the significant series was also considered.

2.2. Study population

In our study we retrospectively included three different groups of patients, seen at the Campus Bio-Medico University of Rome (Departments of Medical Oncology and General Surgery) and affected by: (1) advanced CRC treated with Bevacizumab-containing regimens, (2) advanced CRC treated with not Bevacizumab-con-