

PiRNA signatures of adjacent to tumor tissue as potential biomarkers of gastric carcinogenesis

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According to cancer field effects concepts, tissues adjacent to tumors carry molecular alterations, nevertheless normal appearance. These changes have been described both in genetic and epigenetic levels for different tumors, including gastric cancer. Regardless of these alterations, adjacent to tumor samples are still been used as non-cancer control when aiming to discover new biomarkers. PiRNAs are small non-coding RNA (25-33nt) that interacts with PIWI protein, silences transposable elements maintaining genome stability during early phases of development and also may act on pos-transcription gene regulation. We investigated the piRNA expression profile in paired tumor and adjacent to tumor samples and in gastric sample from non-tumor patients to identify piRNA as cancer biomarkers. A total of 24 samples were analyzed, including eight tumor (GC) and eight adjacent to tumor (AT) paired samples and eight non-cancer gastric tissue (NC). The piRNA was sequenced using Illumina MiSeq platform and statistical analysis was conducted in R using DESeq2 package to identify differentially expressed transcripts ($q\text{-value} \leq 0.05$ and $|\text{fold-change}| > 3$). Assuming adjacent to tumor tissues potentially carry molecular alterations, we compared the expression profile among NC and GC to identify biomarkers capable to differentiate non-cancer to cancer tissue. This analysis identified 12 piRNA differentially expressed, being two under expressed and ten over expressed in GC. In order to evaluate the piRNA expression differences between non-cancer, cancer and adjacent to cancer tissues, the expression profile of AT samples was compared to both NC and GC samples. Twelve piRNA were found differentially expressed when compared to GC and eight to NC. Among the differentially expressed piRNAs, three were found under expressed both in AT and NC samples when compared to GC. In a standard biomarker discovery analysis that compares just AT to GC samples, only these three markers would be able to differentiate a non-cancer tissue from cancer. The remaining nine markers would only differentiate adjacent to tumor tissue from cancer. The standard analysis would also loose nine other potential markers that differentiate non-cancer tissue to cancer. The results were similar to previous findings in miRNA and methylation studies and further support the gastric cancer field effect theory. The consequences of using adjacent to tumor as normal control in molecular analysis involves not only missing biomarkers as well as lose the capability to truly differentiate gastric non cancer from cancer tissues. Thus, the standard strategy to identify molecular biomarkers of cancer should be revised.