

## Regulation of the Fanconi Anemia-BRCA Pathway by MicroRNAs

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**Objective:** DNA crosslinking agents such as cisplatin are widely used anti-cancer drugs. Resistance to these drugs is a major problem for effective cancer therapy. Fanconi anemia (FA) is a cancer-prone genetic disorder characterized by hypersensitivity to DNA crosslinkers. The FA proteins and the breast/ovarian cancer susceptibility genes products (BRCA1, BRCA2) cooperate in a common pathway (the FA-BRCA pathway) required for cellular resistance to DNA crosslinkers. Inhibition of this pathway is therefore an attractive therapeutic strategy to overcome DNA crosslinker resistance. Key proteins in the pathway, such as FANCD2 and RAD51, accumulate at sites of DNA damage and regulate DNA repair, implicated in processing of DNA crosslinks. These nuclear foci, which can be immunocytochemically visualized and quantified, are good markers of the integrity of the pathway.

We hypothesized that the FA-BRCA pathway is regulated by microRNAs. MicroRNAs are 20-24 nucleotide non-coding RNA molecules that post-transcriptionally regulate gene expression. MicroRNAs are involved in biological processes such as cell proliferation, differentiation and apoptosis, and are deregulated in cancer. However, their roles in DNA repair have not been studied yet. The objective of this study is to identify microRNAs regulating the FA-BRCA pathway and modulating chemosensitivity of tumor cells.

**Methods:** We developed a cell-based screening test utilizing decrease of ionizing radiation (IR)-induced FANCD2/RAD51 foci as readout. We screened the human miRIDIAN microRNA Mimics library (Dharmacon), which contains over 400 human microRNA mimics. HeLa cells were transfected with microRNA mimics, irradiated, and immunostained for FANCD2 or RAD51 foci detection. MicroRNA mimics which decreased FANCD2/RAD51 foci were scored as positives.

**Results:** We found that several microRNA mimics inhibit IR-induced foci formation of FANCD2/RAD51. MiR-214, and miR-346 inhibited both FANCD2 and RAD51 foci formation. MiR-15b, miR-122a, miR-138, miR-219, miR-302b, miR-302d, miR-339 and miR-520e preferentially inhibited FANCD2 foci formation, while miR-16, miR-103, miR-105, miR-106a, miR-107, miR-485-3p and miR-506 preferentially inhibited RAD51 foci formation. Interestingly, some of these microRNAs have been already implicated in cancer. MiR-103, miR-106a, miR-107 and miR-214 are known to be upregulated in human pancreatic, colon, prostate, or stomach cancers. MiR-106a is also overexpressed in murine T cell lymphoma induced by retroviral insertion. Therefore, it is possible that overexpression of these microRNAs leads to genomic instability and chemosensitivity in tumors through inhibition of the FA-BRCA pathway.

**Conclusions:** We found that some microRNAs regulate IR-induced foci formation of FANCD2 and RAD51.

**Translational Applicability:** This study suggests the existence of processes regulated by microRNAs in the activation of the FA-BRCA pathway. The elucidation of these processes will further clarify the pathogenesis of FA and may eventually lead to new approaches of the treatment of patients with FA and/or cancer.