**Expression of microRNAs and Target Proteins Show Association with Post-Chernobyl Breast Cancer Development**

Author: Jacob Beckham

Major: Microbiology/Cellular and Molecular Biology

Department of Microbiology and Molecular Genetics, Oklahoma State University, Stillwater, OK 74078, USA

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**Abstract:**

Ionizing radiation is a well‐recognized risk factor for the development of breast cancer. However, it is unknown whether radiation‐specific molecular oncogenic mechanisms exist. Female clean-up workers diagnosed with breast cancer who worked at the Chernobyl site, post meltdown, were investigated for any molecular incongruities when compared to a control group of sporadic breast cancer patients. Increased expression of miRNA‐26b‐5p was found to be associated with radiation exposure in the subjects that were observed. It was also found that the transcriptional target protein of miRNA-26b-5p was downregulated. TRPS1 was characterized in the two radiation‐transformed groups and found that genes that were associated with DNA‐repair, cell cycle, mitosis, cell migration, angiogenesis and EMT pathways were deregulated. The genes associated with TRPS1 were primarily linked to DNA damage response and chromosome segregation. Moreover, the transcriptional interaction partners in the sporadic breast cancers were mostly associated with cell apoptosis. The continued study of how miRNA-26b-5p upregulation and the interrelated TRPS1 downregulation and how it relates to an increase in radiation induced breast cancer formation may lead to the creation of beneficial therapeutic treatments that may halt and/or prevent this specific type of cancer.

**Introduction:**

Breast cancer is one of the most common cancers in women worldwide. Adding to the list of known risk factors such as age, diet, lifestyle, and hereditability; now ionizing radiation has been discovered to be an inducer of cancer. Ionizing radiation takes many forms, including patients that receive radiotherapy or people that are exposed to large scale radiation events such as atomic bomb survivors and nuclear facility meltdown survivors. This is exactly what was discovered after the Chernobyl event in 1986. A significant increase of breast cancer in long-term employed female clean-up workers at the Chernobyl site was recognized when compared to the sporadic breast cancer rates that were not exposed to ion radiation treatments in Ukraine. Specifically, differences in the patient’s expression of certain microRNAs (miRNAs) and their complementary target proteins. miRNAs are 19–25 nucleotides long, noncoding, highly conserved RNA molecules that are known to play an important role in the regulation of gene expression at the post‐transcriptional level. Numerous studies regarding miRNA expression in tumors revealed a significant deregulation in miRNA expression, suggesting that miRNA plays a role in carcinogenesis as a suppressor. Moreover, there has even been an advancement in regard to classifying breast cancer-specific miRNAs into different molecular subtypes; however, the role of miRNAs in radiation‐associated breast cancer has not been researched heavily to the point where the data can be fully implemented and understood.

**Results:**

The experiment utilizes breast cancer cultures from a group of female clean-up workers who were exposed to ionizing radiation from the Chernobyl reactor accident and uses non-ion radiation exposed controls that match for living conditions, tumor type, and age at diagnosis. After researching concurrent literature via the PubMed database, the following miRNAs have data that shows their association to breast cancer development after radiation exposure: hsa‐miR‐26b‐5p, hsa‐miR‐99b‐5p, hsa‐miR‐221‐3p and hsa‐miR‐222‐3p. Various target genes were also compared and the TRPS1 protein was chosen as it was the most conservatively regulated between all miRNA subtypes. qRT‐PCR and differential testing was conducted between the exposed and nonexposed tumor sets which showed hsa‐miR‐26b‐5p was significantly upregulated in the exposed set when compared to the nonexposed tumor set. Assays were conducted to test whether the exposure status was associated with any clinical characteristics of the patients, whereby no significant association could be detected. As for the TRPS1 protein, software‐based quantification revealed a significant downregulation of TRPS1 protein expression from the exposed patients. Histological testing was done to has-miR-26b-5p and TRPS1 to reveal any association with estrogen-receptor status, progesterone-receptor status, or any other histological status wherein none was found. siRNA‐knockdown of TRPS1 was performed in the radiation‐transformed breast cell lines in order to characterize its effect on the transcriptome. The analysis revealed 281 significantly differentially expressed microarray probes relating to 267 different genes (144 downregulated and 137 upregulated). Correlation analysis of validated via qRT‐PCR and mRNA microarray which showed strong correlation for 10 out of the 12 analyzed genes. Of the downregulated genes, many were involved in DNA‐repair, cell cycle, and mitosis while upregulated genes mainly pertained to cell migration, angiogenesis, and EMT pathways. Finally, to validate the data found regarding the effect downregulating TRPS1 The Cancer Genome Atlas (TCGA) was consulted. In total, 12,106 genes showed a statistically significant correlation with TRPS1 expression in sporadic breast cancers. The top 100 correlating genes with regard to sporadic breast cancer were then chosen to be reviewed. After comparison, a significant number of genes were shown to be related to apoptosis related pathways; while the radiation‐associated cell lines showed a significant relation to the process of chromosome segregation and DNA repair.

**Discussion:**

While ionizing radiation is known to be a risk factor for the development of breast cancer, radiation‐specific markers in these tumor types are currently unknown. The identification of has-miR-26b-5p and its effect on the target gene TRPS1 is a clear indicator that progress is being made to identifying radiation-specific markers. Moreover, the regulation differences of hsa‐miR‐26b‐5p between exposed and nonexposed patients has shown that hsa‐miR‐26b‐5p plays a pivotal role in sporadic breast cancer, specifically how its role as a tumor suppressor is affected as the miRNA’s deregulation leads to a negative effect on apoptosis and the suppression of prolific cell growth. This leads to the question of whether radiation‐specific deregulation of hsa‐miR‐26b‐5p affects TRPS1 and its effect on carcinogenesis. As the data suggests, TRPS1 expression was significantly downregulated in exposed cases while the nonexposed sets showed TRPS1 overexpression. In sporadic breast cancer, an upregulated TRPS1 expression was previously observed from the TCGA database findings; additionally, the downregulated TRPS1 results in a more aggressive tumor behavior in radiation-associated breast cancers, which warrants further clarification of the functional role of TRPS1 in a radiation exposure context. With this in mind, TRPS1 has been proposed as a prognostic marker in early stage breast cancer due to its association with improved disease free and overall survival treatment rates. TRPS1 also has potential to act as a negative regulator of EMT and could reduce the metastic potential of breast cancers by transcriptionally suppressing the migration and invasion of other sections of the genome. Similarly, a novel finding of this study is the effect of TRPS1 on the downregulation of DNA repair pathways in radiation-associated cells which suggests the exact radiation-induced effects on the cell’s genomes. This effect on TRPS1 could be an exact cause of how irradiation effects chromosome segregation and genomic instability.

**References:**

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