

The Effects of Space Radiation-changed MiRNAs on Tumorigenesis

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Abstract

Ionizing radiation (IR) including space radiation can transform cells from normal status to tumorigenesis, which is a slow complicated process involving multiple factors and pathways. It is believed that microRNAs (miRNAs) that are small non-coding genes and regulate normal gene expression through binding the 3'untranslated regions of target genes contribute to IR including space radiation-induced tumorigenesis. This review summarizes how IR affects miRNA expression and how the changed miRNA expression in turn affects the status of irradiated cells by describing two samples of miRNA: miR-21 (an onco-miRNA) and miR-34a (a tumor suppressor miRNA) in detail. Understanding the detailed relationship between ionizing radiation and miRNA will help us to find efficient ways to reduce the risk of IR including space radiation-induced carcinogenesis.

Introduction

The data from the Japanese atom bomb survivors demonstrate that low linear energy (LET) ionizing radiation (IR) could increase the incidents for all types of cancers (Preston et al, 2003). High-LET radiation exists in deep space (Zeitlin et al, 2013). Animal data also showed that high-LET IR could be more efficient than low-LET IR at inducing tumorigenesis (Fry et al, 1983, Fry et al, 1985). However, since carcinogenesis is a long-term complicated process, the mechanism underlying carcinogenesis, including IR-induced and high-LET IR-induced carcinogenesis

remains unclear. It is generally believed that carcinogenesis requires either over-activated oncogenes or inactivated tumor suppressor genes. Therefore, we believe that high-LET IR-induced carcinogenesis should be linked to oncogene activation or tumor suppressor inactivation. In this paper, I briefly describe the effects of high-LET IR-induced changes in miRNA expression on tumorigenesis.

MiRNAs could be Oncogenes or Tumor Suppressors

MicroRNAs (miRNAs) are small non-coding RNAs that regulate gene expression at a post-transcriptional level. Long primary transcripts (pri-miRNAs) are processed by Drosha to precursor miRNAs (pre-miRNA) of ~70 nucleotide (nt), translocated to the cytoplasm, and further processed by Dicer or Ago2 to double stranded RNA fragments of 19-22nt (Cheloufi et al, 2010, Cifuentes et al, 2010). One strand of the mature miRNA is incorporated into the RNA-induced signaling complex (RISC), which delivers it to its target mRNAs. Base pairing with the 3' untranslated region (UTR) of target mRNAs promotes degradation or translational blockage depending on the degree of complementarity. The transcription of pri-miRNAs is RNA polymerase II-dependent and is under similar regulatory controls (eg. transcription factors, epigenetic mechanisms) as protein coding genes.

Most human genes are regulated by at least one miRNA (Friedman et al, 2009). Considering that ~1% of the human genome is devoted to miRNA genes (Bartel, 2004), and each miRNA has multiple mRNA targets, the potential impact of altered miRNA levels is conceivably enormous. Aberrant regulation of miRNAs is common in human cancers (Davalos and Esteller, 2010, Iorio et al, 2010), and can result from defects in the miRNA processing machinery (Drosha, Dicer, Ago2), constitutive activation of oncogenic transcription factors, and gene deletions,

amplifications, or translocations. miRNAs could be oncogenes through targeting tumor suppressors or could be tumor suppressors through targeting oncogenes. However, there are two factors that we should pay attention to. One factor is that one miRNA could target both oncogenes or tumor suppressors at the same time since one miRNA could target multiple genes. At such conditions, the miRNA acting as an oncogene or tumor suppressor should depend on the balance of the neutralizing effects. The other factor is that one miRNA in one cell line from one type of tissue is a tumor suppressor/oncogene could be an oncogene/tumor suppressor in other types of cell lines from other types of tissue since miRNA expression is tissue dependent (Ro et al, 2007, Rosenfeld et al, 2008).

Ionizing Radiation Could Change miRNA Expression

Many studies have reported that IR including both low-LET and high-LET could change miRNA expression patterns in different types of human or mouse cells (Chaudhry et al, 2010, Khan et al, 2013, Maes et al, 2008, Templin et al, 2011, Wagner-Ecker et al, 2010). Among the IR-induced miRNA changes, some are up-regulated and some are down-regulated. In general, the change in miRNA expression is temperate, time and dose dependent, most of them disappeared at 24 h after IR (Maes et al, 2008). As mentioned above, since miRNA expression depends on the different tissue origin, it is difficult to get one conclusion for the IR-changed miRNA expression profiles of different cell lines from different reports.

It remains unclear how IR induces the change in miRNA expression. It is known that p53, an IR-inducible transcription factor, is a positive regulator of miR-34a (Chang et al, 2007, He et al, 2007, Raver-Shapira et al, 2007), suggesting that IR-could promote miR-34a expression through stimulating p53. However, it is also known that p63, a member of the p53 family, negatively

regulates miR-34a expression (Antonini et al, 2010). These results indicate that the miR-34a level in irradiated cells depends on the balance of p53 and p63. My group has studied the mechanism by which IR-induced miR-21 up-regulates in human hepatocytes (Zhu et al, 2010). We showed in this study that at an earlier time after IR (< 2 h), the transcription factor, Ap1, was over-activated and at a later time after IR (> 2 h), the transcription factor, Stat3 (and its upstream regulator, EGFR) , was over-activated in the cells (Zhu et al, 2010). We also showed that after knocking down Ap1 or Stat3, the IR-up-regulated miR-21 level decreased. In addition, we showed that the up-regulation of miR-21 could be maintained for up to one year in a brain from whole body high-LET irradiated (0.5 Gy) mice, which was associated with the up-regulation of EGFR (Shi et al, 2012). These data strongly support that IR-induced up-regulation of miR-21 is due to IR stimulating the transcription factors of miR-21. In the near future, more studies are needed to also investigate the mechanisms underlying IR-changed miRNA expression in addition to to studying the miRNAs' targets, which will allow us to better understand the effects of the miRNA network on IR-induced carcinogenesis. In the following paragraphs, I describe the effects of two miRNAs: miR-21 (an oncogene) and miR-34a (a tumor suppressor) on IR-induced carcinogenesis.

Link between IR-induced miR-21 Up-regulation and IR-induced Carcinogenesis

Although in general, miRNA expression has tissue-specificity, miR-21 is a unique miR that has been reported to show an up-regulation in almost all types of human tumors, suggesting that miR-21 expression has no clear tissue-specific choice. The over-expression of miR-21 is associated with advanced stage, lymph node metastasis and a poor prognosis (Asangani et al, 2008, Chan et al, 2005, Hwang et al, 2010, Liu et al, 2010, Seike et al, 2009, Volinia et al, 2006, Wickramasinghe et al, 2009). This association of miR-21 over-expression and tumor progress is

involved in that miR-21 targets the tumor suppressors PTEN, PDCD4 and TPM1 (Lu et al, 2008, Qi et al, 2009, Si et al, 2007). It has been demonstrated that over-expressing miR-21 resulted in tumorigenesis and knockout miR-21 resulted in less tumorigenesis in the miR-21 knock-in or knockout mouse models (Hatley et al, 2010, Ma et al, 2011, Medina et al, 2010). These results indicate that miR-21 is an oncogene. MiR-21 is upregulated in response to ionized radiation (Chaudhry et al, 2010, Wagner-Ecker et al, 2010), particularly to high-LET irradiated human hepatocytes (Zhu et al, 2010). We also showed that over-expression of miR-21 in human hepatocytes (non-tumorigenesis) could make cells become tumorigenesis in nude mice, and the tumor size was larger when the cells were irradiated with 0.5 Gy (particularly with high-LET IR) before they were injected into the nude mice (Zhu et al, 2010). These results suggest that IR and up-regulation of miR-21 have a synergistic effect on tumorigenesis, supporting that IR-induced up-regulation of miR-21 contributes to IR-induced carcinogenesis.

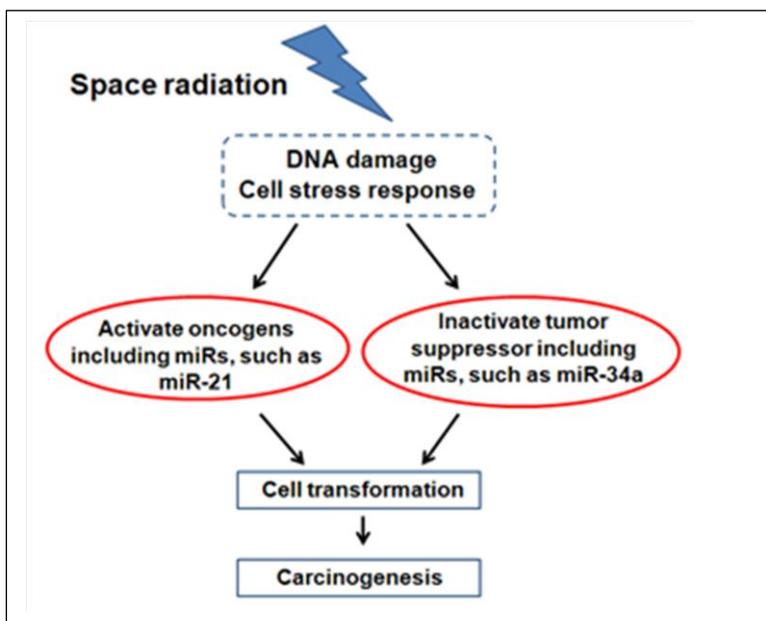


Figure 1. Outline of space radiation-induced carcinogenesis through modulating miRNAs.

To study the mechanism by which up-regulation of miR-21 contributes to IR-induced carcinogenesis, we identified new targets of miR-21, SODs (Zhang et al, 2012) that play an important role to clean endogenous and exogenous reactive oxygen species (ROS). It is known that IR-could stimulate ROS generation that contributes to tumorigenesis. We identified that SOD3 and TNF- α (an upstream transcription factor of SOD2) as the key targets of miR-21 for mediating the ROS levels in irradiated cells (Zhang et al, 2012). We found that IR increased the ROS levels through up-regulating miR-21 that directly inhibited SOD3 and indirectly inhibited SOD2 (through inhibiting TNF- α), which promoted the cell transformation that is an initial key step for cells from normal development to an oncogenic stage (Zhang et al, 2012). Multiple pathways such as cell growth control, apoptosis, senescence, and DNA damage response, contribute to cell transformation (Dimri et al, 2005); although, the whole picture remains unclear. Our study demonstrates that the miR-21 mediated ROS level through targeting SODs contributes to IR-induced cell transformation. This is an example for an oncogenic miRNA to involve IR-induced tumorigenesis. Next, there is another example for a tumor suppressor miRNA to involve IR-induced tumorigenesis.

Link between IR-induced miR-34a Down-regulation and IR-induced Carcinogenesis

miR-34a as a tumor suppressor (Cole et al, 2008, Dalgard et al, 2009, Di Leva and Croce, 2013, Kasinski and Slack, 2012, Tazawa et al, 2007, Yin et al, 2013) is one of the down-regulated miRNAs in high-LET transformed human epithelial cells (Ng et al, 2013). To study the effects of the down-regulation of miR-34a on IR-induced cell transformation, we identified OCT4 as a novel target of miR-34a (Ng et al, 2013). OCT4 is one of the major transcriptional factors that play a key role during the induced pluripotent stem cell (iPSC) process (Takahashi and Yamanaka, 2006, Takahashi et al, 2007). Since human cancer stem cells (hCSCs) share

many characteristics with human embryonic stem cells (hESCs). It is possible that as normal human cells transform into an oncogenic stage they share some similar mechanisms with the iPSC process. p53 stimulates miR-34a expression (Antonini et al, 2010, Chang et al, 2007, He et al, 2007, Raver-Shapira et al, 2007), which plays an important role in stimulating stem cell differentiation and inhibiting tumorigenesis. As a member of the p53 family, p63 has many different functions from p53 (Crum and McKeon, 2010), that includes inhibition of the miR-34a expression (Antonini et al, 2010) and maintaining stem cell characteristics (Blanpain and Fuchs, 2007, Koster et al, 2004, Lin et al, 2005, McKeon, 2004, Pellegrini et al, 2001, Senoo et al, 2007, Su et al, 2009, Wang et al, 2011).

Through studying the high-LET IR-transformed cells, we revealed a novel functional link among p53 or p63 and miR-34a to target *OCT4*, as well as *OCT4* feedback to target p53 or p63 with different consequences, which significantly affects cell transformation (Ng et al, 2013). We found that *OCT4* as a target of miR-34a could inhibit p53 but stimulate p63 expression (Ng et al, 2013). We showed that p53 and p63 have opposite effects on human cell transformation through regulating miR-34a/*OCT4* and, in turn, are affected by *OCT4* (Ng et al, 2013). Our results detail the functional relationships among these factors and demonstrate that the balance of two functional loops, p53-miR-34a-*OCT4*-p53 and p63-miR-34a-*OCT4*-p63, are decisive in high-LET IR-induced cell transformation. These results provide strong evidence for the similarity between iPSC and oncogenic cell transformation.

In summary, IR including space radiation (high-LET IR) could induce tumorigenesis. Although IR-induced tumorigenesis is a complicated process that involves multiple factors and pathways, it is clearly that IR-changed miRNA expression contributes to IR-induced

carcinogenesis (Figure 1). Understanding the detailed mechanism will help us to find efficient ways to reduce the risk of IR including space radiation-induced carcinogenesis.

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