3 - 53 $p53$ Family Regulate MicroRNA Expression and Biogenesis in Cellular Response to IR*

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$p53$ mainly exerts its function through transcriptional regulation of its targets. In response to various stress signals, including the DNA damage response after IR, ATM phosphorylates $p53$ leads to its dissociation from MDM-2, an inhibitor of $p53$, and accumulation of $p53$[1]. The accumulation of $p53$ leads to the transcriptional activation of its target genes and initiates various cellular responses. Several studies have demonstrated that miRNA expression and biogenesis are controlled by $p53$ in cellular response to IR.

The first discovery connecting $p53$ to the regulation of miRNAs expression is the identification of the miR-34 family. MiR-34s can be induced by IR in vitro and in vivo whose expression is precisely correlated with $p53$ status. Predicted gene structure for miR-34 family shows that the promoter regions included a palindromic sequence that matched the canonical $p53$ binding sites. The induction of miR-34s by $p53$ in cellular response to IR is further confirmed by other groups.

The *let*-7 family is other miRNAs that are regulated by $p53$. Saleh *et al.* describe that *let*-7a and b are transcriptionally repressed by $p53$ after IR. $p53$ can directly bind the region upstream of *let*-7a and $b$leading to its expressional repress. The expression of *let*-7a and b not only depends on functional $p53$, but also depends on IR-induced ATM signaling upstream of $p53$. However, there are inconsistencies among various cell lines as to whether *let*-7 $miRNAs$ were up- or down-regulated upon IR. The detected differential expression of these $miRNAs$ might be explained by following facts. On one hand, microRNAs are expressed in a tissue- or cell type-specific manner, the differences in species, model system, cell type, and irradiation conditions *i.e.*, they are differentially expressed at radiation types, time points, and/or doses that are quite different from each other. On the other hand, some $miRNAs$ may belong to multiple "response networks" that are activated by different cellular stimuli. Moreover, the limited power of our analysis to detect differentially expressed $miRNAs$ with low fold changes may have prevented the detection of these $miRNAs$ in additional irradiation conditions. These studies suggest that $p53$, activated by DSBs caused by IR, play a critical role in the regulation of *let*-7 family $miRNAs$ expression.

Moreover, miR-192, miR-194 and miR-215 are other $miRNAs$ that appeared to be regulated by $p53$ in cellular response to IR. Several studies reveal that miR-192, miR-194 and miR-215 are significantly up-regulated by IR in different normal and cancer cell lines. IR-caused DNA damage promotes the $p53$-dependent up-regulation of miR-192, miR-194 and miR-215. The genomic region around the miR-194/miR-215 cluster contains a putative $p53$-binding element, suggest that the cluster are activated by $p53$ at transcriptional level.

$p53$, as a transcriptional factor and target of ATM, not only regulates miRNA expression at transcriptional level but regulates miRNA biogenesis at post-transcriptional level in cellular response to IR. Some of $miRNAs$, including miR-16-1, miR-143 and miR-145, are up-regulated in a $p53$-dependent and $p68/p72$-dependent manner in the DDR. $p53$ can interact with the Drosha/DGCR8 processing complex through an association with RNA helicase$p68$ (DDX5) and$p72$ (DDX17). A direct interaction between $p53$ and $p68/p72$ facilitates $p53$ promoting of miRNA processing from pri-$miRNAs$ to pre-$miRNAs$. $p53$ mutants disrupt a functional assembly between Drosha complex and $p68$, resulting in attenuation of miRNA processing activity. Similar to $p53$, $TAp63$ could bind to and transactivate the promoters of Dicer and miR-130b, and direct regulated the biogenesis and expression of $miRNAs$. $p63/p73$ is also noted that function as both positive and negative regulators of the miRNA transcription and processing components and regulate the expression and biogenesis of multiple microRNAs.

Now, it currently remains largely unknown about whether and how $p53$ family regulate the miRNA biogenesis in cellular response to IR. For instance, there is no direct evidence that confirms $p53/p63/p73$ regulates miRNA processing and maturation in cellular response to IR. Whether and how $p53/p63/p73$ regulates $miRNAs$ processing in cellular response to IR? Moreover, whether and how $p53/p63/p73$ modulate the transportation of pre-$miRNAs$ from the nucleus to cytoplasm after IR? Whether IR-induced DNA damage affects the degradation or modification of $miRNAs$? How $p53/p63/p73$ regulates the degradation of $miRNAs$? These questions should be further confirmed and elucidated in the future research.

Furthermore, we recently discovered that there was a differential ΔNp73 expression in response to different LET radiations, and downregulated ΔNp73 expression play a critical role in promoting cycle arrest and apoptosis in Hela

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cells. ΔNp73, as an antagonist to $p53/p63/TAp73$, whether and how the downregulated ΔNp73 expression affects the miRNA biogenesis in cellular response to IR remains unknown. It will be interesting to clarify the relationship of ΔNp73 expression and miRNA biogenesis in cellular response to different LET irradiation. We should pay close attention to discover the effect of different LET irradiation on $miRNAs$ expression and biogenesis and the regulatory mechanism of biogenesis employing the HIRFL (Heavy Ion Research Facility of Lanzhou, Institute of Modern Physics, and Lanzhou, China)[2].

In summary, IR-induced DSBs directly activate $p53$ or ATM phosphorylates $p53$ to mediate $miRNAs$ transcription by binding the promoter regions of $miRNA$ genes. $p53/p63/p73$ also can interact with Drosha/DGCR8 complex through $p68$ and $p72$ to enhance the $miRNAs$ expression. Whether $p53/p63/p73$ influence $miRNAs$' transportation, degradation and RISC assembly is unclear and need further investigation.

References

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3 - 54 DNA-PKcs Deficiency Inhibits Glioblastoma Cell-Derived Angiogenesis after Ionizing Radiation *

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DNA-dependent protein kinase catalytic subunit (DNA-PKcs) plays a critical role in non-homologous end-joining repair of DNA double strand breaks (DSB) induced by ionizing radiation (IR) [1]. Little is known, however, regarding the relationship between DNA-PKcs and IR induced angiogenesis; thus, in this study we aimed to further elucidate this relationship. Our findings revealed that lack of DNA-PKcs expression or activity sensitized glioma cells to radiation due to the defective DNA DSB repairs and inhibition of phosphorylated Akt Ser473. Moreover, DNA-PKcs deficiency apparently mitigated IR-induced migration, invasion and tube formation of human microvascular endothelial cell (HMEC-1) in conditioned media derived from irradiated DNA-PKcs mutant M059J glioma cells or M059K glioma cells that have inhibited DNA-PKcs kinase activity due to the specific inhibitor NU7026 or siRNA knockdown(Fig. 1). Moreover, IR-elevated vascular endothelial growth factor (VEGF) secretion was abrogated by DNA-PKcs suppression. Supplemental VEGF antibody to irradiated conditioned media was negated enhanced cell motility with a concomitant decrease in phosphorylation of the FAK^{Try925} and Src^{Try416}. Furthermore, DNA-PKcs suppression was markedly abrogated in IR-induced transcription factor hypoxia inducible factor-1α (HIF-1α) accumulation, which is related to activation of VEGF transcription.

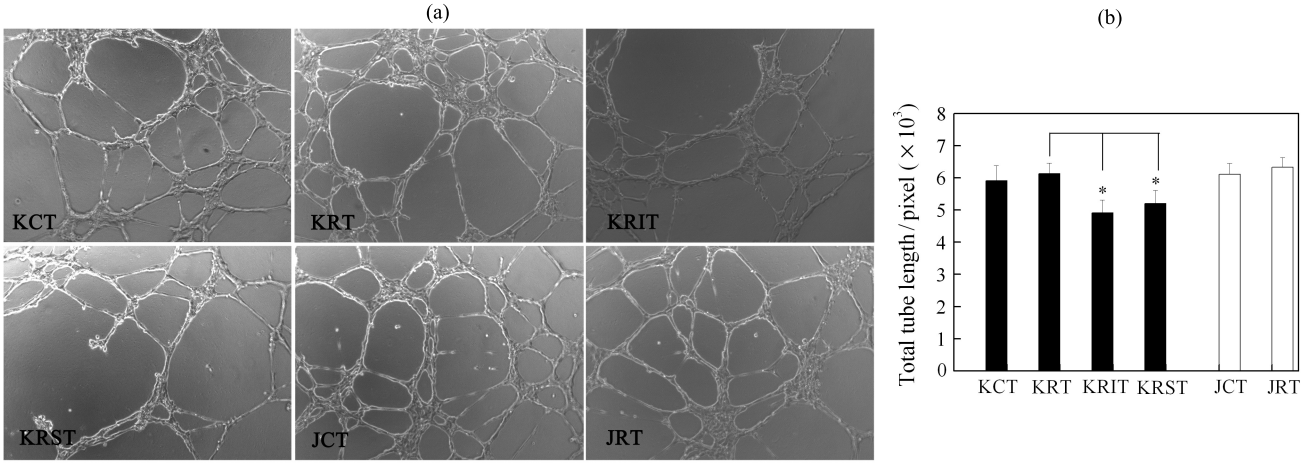

Fig. 1 Effects of conditioned media derived glioma cells with different DNA-PKcs kinase activity on tube formation. (a) Representative photographs of HMEC-1 endothelial cell culture in Matrigel (8 h). (b) Tube formation is expressed as total tube length. KCT, TCM from M059K cells; KRT, ICM from M059K cells; KRIT, ICM from M059K cells pretreated with the DNA-PKcs inhibitor NU7026; KRST, ICM from M059K cells pretreated with DNA-PKcs siRNA; JCT, TCM from M059J cells; JRT, ICM from M059J cells.