Final Report

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Title: Characterization of the role of Fhit in maintenance of genomic integrity following low dose radiation, *in vivo* and *in vitro*

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Description:

This grant started on July 1, 2005 at Thomas Jefferson University and finished on May 30, 2010 at Emory University. Dr. Ya Wang, the PI of this grant relocated from Thomas Jefferson University to Emory University on September 1, 2008. This final report includes the work that was carried out at both Thomas Jefferson University and Emory University.

The major goal of this study is to determine the effects of the Fhit pathway on low dose ($\leq$ 0.1 Gy) ionizing radiation (IR)-induced genetic instability. Reduction of Fhit protein expression is observed in most solid tumors particularly in those tumors resulting from exposure to environmental carcinogens. Therefore, characterization of the role of the Fhit-dependent pathway in preventing low dose IR-induced genetic instability will provide useful parameters for evaluating the low dose IR-induced risk of mutagenesis and carcinogenesis. We pursued 3 specific aims to study our hypothesis that the Fhit-dependent pathways maintain genomic integrity through adjusting checkpoint response and repair genes expression following low dose IR. Aim 1: Determine whether Fhit interaction with RPA is necessary for Fhit to affect the cellular response to low dose IR. We combined the approaches of *in vitro* (GST pull-down and site-directed mutagenesis) and *in vivo* (observing the co-localization and immunoprecipitation of Fhit and RPA in *Fhit* knock out mouse cells transfected with mutant Fhit which has lost ability to interact with RPA *in vitro*). Aim 2: Determine the role of genes whose expression is affected by Fhit in low dose irradiated cells. We analyzed the distinct signature of gene expression in low dose irradiated Fhit-/- cells compared with Fhit+/+ cells by combining microarray, gene transfection and siRNA approaches. Aim 3: Determine the role of Fhit in genetic susceptibility to low dose IR *in vivo*. We compared the gene mutation frequency and the fragile site stability in the cells isolated from the Fhit+/+ and Fhit-/- mice at 1.5 years following low dose IR. These results determine the role of the Fhit-dependent pathway in maintaining genomic integrity *in vitro* and *in vivo*, which provide a basis for choosing surrogate markers in the Fhit-dependent pathway to evaluate low dose IR-induced risk of mutagenesis and carcinogenesis.

Major works:

Our laboratory for the first time identified the role of Fhit in DNA damage induced checkpoint, which is shown in several presentations and published manuscripts. Meanwhile, when we worked on aim 1 to study whether Fhit interaction with RPA is necessary for Fhit to affect the cellular response to low dose IR, we found that Fhit is not directly interacting with RPA and the reason why we showed the interaction in our preliminary results is that the RPA directly binds to protein A. This non-specific binding is very easy to lead to wrong conclusions. Based on these results, we published our discovery to alert scientists in their immunoprecipitation experiments.

We found that at our experiment condition low dose IR ($\leq$ 0.1 Gy) induced adapted effects could prevent IR-induced mutation *in vitro*. We previously reported that Fhit, a gene related to human tumor progression, could prevent UV-induced *HPRT* mutation, suggesting that Fhit prevents DNA damage-induced mutation. Therefore, we were interested in investigating whether Fhit prevents IR-induced *HPRT* mutation and whether Fhit plays any role in low dose induced adapted effects on preventing *HPRT* mutation. For this purpose, we established human cell lines with or without Fhit expression. We examined IR dose response of *HPRT* mutation frequencies

and low dose of IR (0.1 Gy) induced adapted effects on preventing mutation in these cell lines. The results show that low dose of IR (0.1 Gy) does not increase *HPRT* mutation frequencies in these cell lines. Fhit prevents high dose IR ($\geq$ 2 Gy) induced mutation as it does prevent UV induced mutation. However, low dose of IR (0.1 Gy) induced adapted effects prevent both high doses of IR and UV induced mutation in both the cells with and without Fhit expression. These results indicate that low dose of IR-induced adapted effects on preventing mutation are Fhit independent. This work is described in one manuscript that is published.

We found that at our experiment condition low dose IR ($\leq$ 0.1 Gy) induced adapted effects could prevent IR-induced tumor *in vivo*. Low-dose ($\leq$ 0.1 Gy) radiation could reduce high-dose induced damage including tumorigenesis. However, it remains unclear whether multi-exposure to low-dose radiation at a high dose rate has any risk for increasing tumorigenesis, and whether Fhit plays any role in the process. The purpose of this study is to investigate the effects of multi-exposure to low-dose radiation at a high dose rate on tumorigenesis, and the role of Fhit in it. We irradiated $Fhit^{+/+}$ and $Fhit^{-/-}$ mice with 1 Gy/1 or 0.1 Gy x 10 exposures at a dose rate of 1 Gy/min, sacrificed the mice at 1.5 years after radiation and observed multi-organ tumorigenesis. The results showed that although the spontaneous tumorigenesis in these mice was relatively high, 1 Gy/1-exposure dramatically increased the tumorigenesis including lung and liver tumor. $Fhit^{-/-}$ mice showed more tumorigenesis than $Fhit^{+/+}$ mice after 1 Gy/1-exposure. However, 0.1 Gy x 10 exposures did not increase tumorigenesis, and there was no statistical difference in tumorigenesis between $Fhit^{+/+}$ mice and $Fhit^{-/-}$ mice following 0.1 Gy x 10 exposures. Our results suggest that 0.1 Gy, even after multiple exposures, does not increase tumorigenesis, and Fhit could prevent high-dose radiation-induced tumors but has no effect in a low-dose environment. This work is described in one manuscript that is published.

In addition, we further explored the mechanism underlying the low dose radiation-induced adapted response. Low dose ($\leq$ 0.1 Gy) radiation-induced adaptive responses could protect cells from high challenge dose radiation-induced killing. The protective role is believed to promote the repair of DNA double strand breaks (DSBs) that are a severe threat to cell survival. However, it remains unclear which repair pathway, homologous recombination repair (HRR) or non-homologous end-joining (NHEJ), is promoted by low dose radiation. To address this question, we examined the effects of low dose (0.1 Gy) on high challenge dose (2-4 Gy) induced killing in NHEJ or HRR deficient cell lines. We showed that 0.1 Gy reduced the high dose radiation-induced killing for wild type or HRR deficient cells, but enhanced the killing for NHEJ deficient cells. Interestingly, low dose radiation also enhanced the killing for wild type cells exposed to high challenge dose radiation with high-linear energy transfer (LET). Because it is known that high-LET radiation induces an inefficient NHEJ, these results support that the low dose radiation-stimulated protective role in reducing high challenge dose (low-LET)-induced cell killing might depend on NHEJ. In addition, we showed that low dose radiation activated the DNA-PK catalytic subunit (DNA-PKcs) and the inhibitor of DNA-PKcs destroyed the low dose radiation-induced protective role. These results suggest that low dose radiation might promote NHEJ through the stimulation of DNA-PKcs activity and; therefore, increase the resistance of cells to high challenge dose radiation-induced killing. This work is described in one manuscript that is published.

Publications and Presentation:

1. Lu, L, Hu, B. and Wang Y. Fhit affects cell response to low dose ionizing radiation. 98th Annual Meeting of American Association for Cancer Research, Los Angeles. April 14-April 18, 2006.
2. Lu L, Hu, B. and Wang Y. The role of Fhit in low dose ionizing radiation-induced checkpoint response is through affecting the RPA pathway. 53rd Annual Meeting of Radiation Research Society, Philadelphia, November 5- 8, 2006.
3. Ishii, H., Wang, Y. and Huebner, K. A Fhit-ing role in the DNA damage checkpoint response. Cell Cycle, 6: 1044-1048. 2007.
4. Lu, L. Hu, B. and Wang, Y. Low dose induced adapted effects on *HPRT* mutation are Fhit independent. Present at The Low Dose Workshop, Washington DC January 21-23, 2008,
5. Lu, L. and Wang, Y. Immunoprecipitation alert: DNA binding proteins directly bind to protein A/G without any antibody as the bridge. Cell Cycle, 7: 417-418, 2008.
6. Pichiorri, F., Ishii, H., Okumura, H., Wang, Y. and Huebner, K. Molecular parameters of genome instability: roles of fragile genes at common fragile sites. J Cell Biochem, 104:1525-1533, 2008.
7. Yu, X. Lu, L., Wen, S. and Wang, Y. Fhit prevents radiation induced carcinogenesis. Low Dose Radiation Research Program Investigators' Workshop VIII, Washington, DC April 6-8, 2009.
8. Lu, L. Hu, B. and Wang, Y. Low dose induced adapted effects on *HPRT* mutation are Fhit independent. Int. J. Radiat. Biol. 85:532-537, 2009.
9. Yu, X., Lu, L., Wen, S. and Wang, Y. The effects of Fhit on tumorigenesis after multi-exposure to low dose radiation. IJCEM, 2, 348-353, 2009.
10. Yu, X., Wang, H., Wang, P. Guida, P., Chen, BPC and Wang, Y. The Ku dependent non-homologous end-joining pathway contributes to low dose radiation-stimulated cell survival. Low Dose Radiation Research Program Workshop, Washington DC April 12-14, 2010.
11. Yu, X., Wang, H., Wang, P. Guida, P., Chen, BPC and *Wang, Y. The Ku dependent non-homologous end-joining pathway contributes to low dose radiation-stimulated cell survival. J Cell Physiol. DOI: 10.1002/jcp.22342, 2010

Budget:

We have finished the work with the total funded budget.