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14. ABSTRACT  The hMOF protein is a chromatin-modifying factor. Chromatin structure plays a critical role in gene expression. Since hMOF has a chromodomain region as well as acetyl transferase activity, its inactivation can influence modification of chromatin during DNA metabolism. The proposed experiments of this grant proposal will determine functions of hMOF gene. This will be achieved by generating isogenic cells with and without hMOF function. Both in vivo and in vitro experiments will be performed to determine the function of hMOF in context with radio responsiveness and oncogenic transformation. If hMOF proves to be involved in the radio responsiveness and neoplastic transformation, then the clinical implications of this proposal are highly significant. It may, in the future, be prudent to screen each breast cancer patient prior to any final therapeutic decision. This will be accomplished through the use of quantitative RT-PCR and the test results can be obtained within a day. There are several benefits of identifying an individual's normal tissue with loss of hMOF gene expression. First, it will allow us to prospectively identify the sensitive subset of patients. Second, the radiosensitive patients will be taken for an alternative therapy if exist and would be spared a great deal of suffering. Third, it will be possible that once we identify a subset of patients that show a genetic basis of radiation sensitivity, the radiation dose to the remaining breast patients could be increased to be more effective for local tumor control. Fourth, it will provide health professionals a molecular diagnostic approach to predict the suitability of an individual for radiotherapy.					
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## Introduction:

Most patients with breast cancer tolerate radiotherapy well with only limited acute, reversible adverse effects. However, about 5% of patients experience severe, delayed complications such as skin pigmentation changes, subcutaneous fibrosis, rib fractures, cardiac disease, pulmonary fibrosis, second primary cancer (specifically esophageal squamous-cell carcinoma as well as adenocarcinoma) and other complications, which manifest several years after treatment with ionizing radiation. Epidemiological studies have shown that irradiation of the breast especially among young women, increases the risk for subsequently developing breast cancer. It might thus be expected that genes that are known to influence radiation sensitivity may be associated with the radiotherapy related adverse effects. The human genes that have been found to be responsible for ionizing radiation sensitivity are *ATM* (ataxia telangiectasia mutated), *BRCA1*, *BRCA2*, *NBS1*, etc. Mutations in *BRCA1* and *BRCA2* contribute to about 15% of familial breast cancer risk and their contribution to sporadic breast cancer is very low. In such cases, genes frequently altered in the general population, e.g., *ATM* may be an important risk factor. However, screening for *ATM* mutations in sporadic breast cancer cases has not revealed the magnitude of involvement of the *ATM* gene expected. Since *ATM* as well as *BRCA1* have been reported to interact with chromatin modifying factors, it is possible that such factors may be involved in the radiation-induced morbidity. Therefore, there is a need for the identification of chromatin modifying factors involved in ionizing radiation sensitivity, genomic instability and carcinogenesis.

## Body

### Specific Aims:

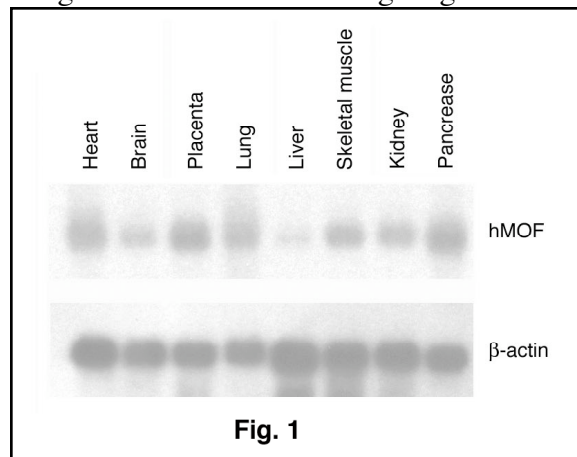
The goal of this proposal is to understand the mechanisms underlying radiosensitivity. Two specific questions are being addressed in this grant application: (1) Whether hMOF is involved in ionizing radiation (IR) response and; (2) Whether hMOF is involved in pathobiology of the breast cancer. We proposed to complete the following aims: (1) To determine whether mutations in the *hMOF* gene correlate with ionizing radiation sensitivity. (2) To generate MOF knockout mice in order to determine the pathobiology of gene. (3) To determine whether ionizing radiation enhances neoplastic transformations in mouse embryonic fibroblasts of MOF knockout mice. MOF knockout mice will also be examined for spontaneous as well as IR-induced tumor formation.

### Studies and Results at the end of third year of funding:

During the third year, we have addressed the specific aim 2. This specific aim allowed us to determine the interaction of hMOF with ATM and generate mouse MOF targeting vector for generating mouse knock out mice.

**Task2.** (a) To generate MOF knockout mice in order to determine the pathobiology of gene:

To assess the contribution of hMOF in mammalian development, we first determined the expression status of hMOF using a multi-tissue Northern blot analysis. Expression of hMOF mRNA was found in all tissues (Fig. 1). To understand the genopathology of hMOF, we have cloned and sequenced a full-length mouse *Mof* cDNA. To isolate an isogenic *Mof* mouse gene for construction of the targeting vector,



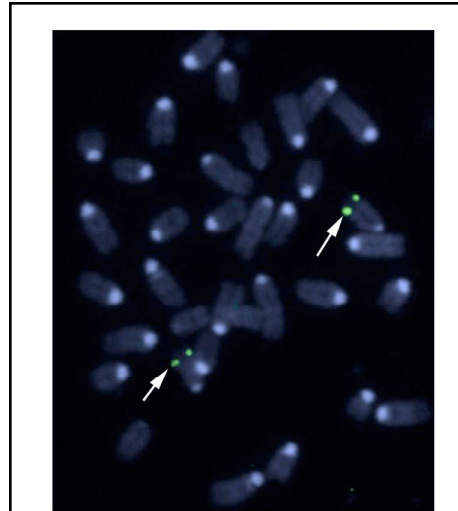
**Fig. 1.** *hMOF* expression levels: Autoradiograph showing Northern blots from normal human multiple tissues.

**Fig. 2.** The *mMOF* gene was localized by FISH analysis to distal chromosome 7 (arrow) by the FISH procedure.

**Fig. 3.** Organization of the wild-type gene, the targeting construct, and the structure of the locus gene targeting. *Bgl*II (B), *Eco*RI (E), *Xba*I (X), *Sal*I (S).

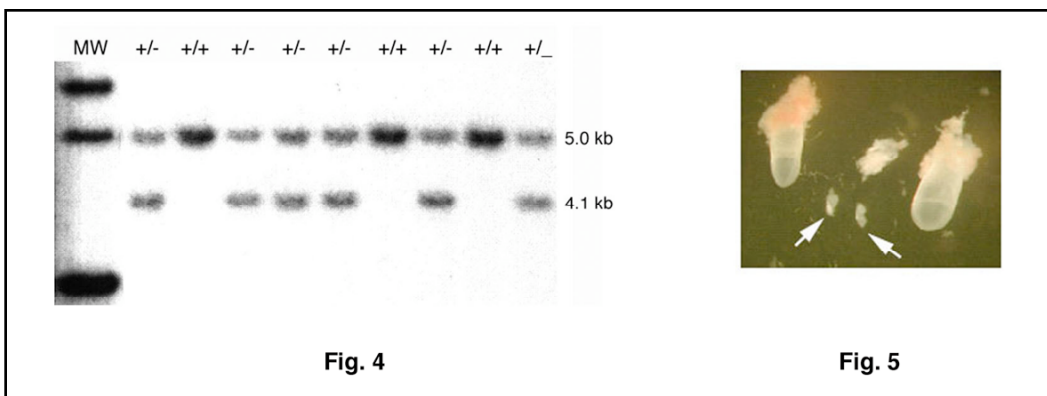
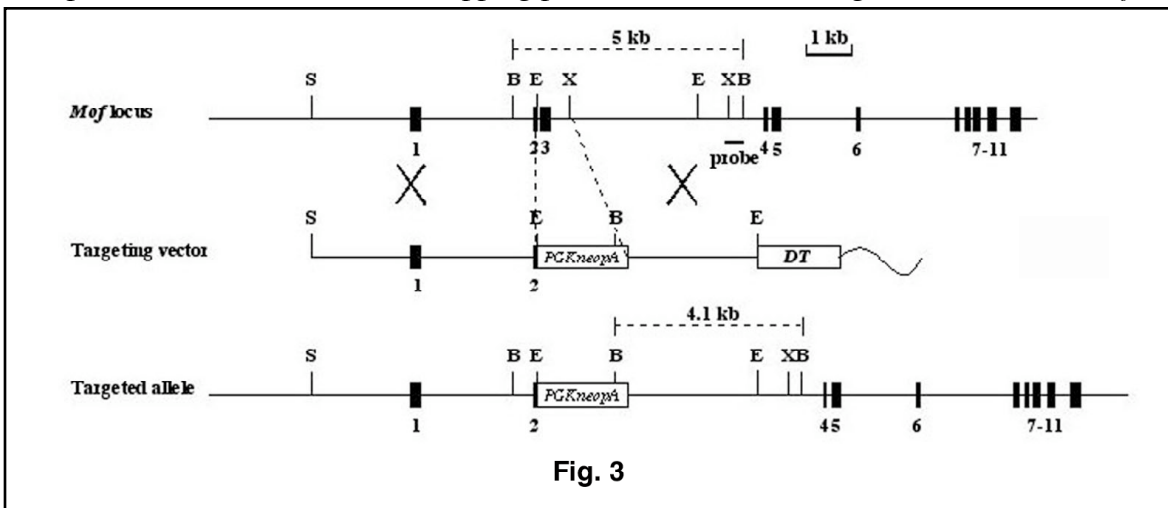
**Fig. 4.** Southern analysis of tail DNA digested with *Bgl*II and hybridized with a probe located outside the targeting construct (indicated in Fig. 3).

**Fig. 5.** Gross morphology of embryos. Two normal (left and right) and three mutant embryos dissected at E7.5 (arrow).



**Fig. 2**

we screened a genomic  $\lambda$  phagelibrary from the mouse strain 129/Sv (Stratagene) using *Mof* cDNA as a probe and obtained three overlapping positive clones containing exons 1-4 of the *Mof*



gene. We identified 4 and 9 kb *Eco*RI fragments of *Mof*. The genomic mouse *MOF* which is localized on chromosome 7 (Fig. 2) A gene-targeting vector was designed to inactivate *mMof* gene after homologous recombination (Fig. 3, 4). Mating between *mMOF* heterozygotes yielded the frequency of wild type (WT), heterozygous (*mMof*<sup>+/-</sup>) and homozygotes

(mMof+/-) offspring in the ratio of 1:2:0 indicating that mMof is required for normal mouse development. mMof+/- mice were embryonic lethal at day 6.5 (Fig. 5).

(b) Involvement of hMOF in ATM function:

We have determined that hMOF is associated with the ATM (ataxia-telangiectasia mutated) protein. Cellular exposure to ionizing radiation (IR) enhances hMOF-dependent acetylation of its target substrate, lysine 16 (K16) of histone H4, independent of ATM function. Blocking the IR-induced increase in acetylation of histone H4 at K16, either by expression of a dominant negative mutant hMOF or by RNAi-mediated hMOF knockdown, resulted in decreased ATM autophosphorylation, ATM kinase activity, phosphorylation of downstream effectors of ATM and DNA repair while increasing cell killing. In addition, decreased hMOF activity was associated with defective telomere metabolism and loss of the cell cycle checkpoint response to DNA double strand breaks (DSBs). Over-expression of wild-type hMOF yielded the opposite results; increased cell survival and enhanced DNA repair after IR exposure. These results suggest that hMOF influences the function of ATM. The details of these results have resulted in a very comprehensive publication (Gupta et al., 2005; Mol Cell Biol 25:5292-5305).

➤ **Key Research Accomplishments**

- We cloned cDNA and genomic mouse MOF gene.
- We made targeting vector to generate the Mof knockout mice.
- We generated mice heterozygous for MOF gene.
- We established the interaction between hMOF and ATM protein.
- We determined hMOF inactivation abrogates ATM functions

**c. Reportable Outcomes**

1. Cloned cDNA and genomic DNA of mouse MOF gene.
2. Generated mouse MOF heterozygote mice.
3. MOF inactivation results in embryonic lethality.
4. Determined the influences of hMOF on ATM function.

**d. Conclusions: Plans for next year (2005-2006):**

During the fourth year, we will complete the work proposed under task 3.

**Task 3:** (a) The global ablation of Mof function in the mouse resulted in early embryonic lethality, we will construct a targeting vector for conditional mutagenesis, which will allow the global and the tissue-specific inactivation of *Mof*. Currently the *cre/loxP* strategy is probably the most applied system of conditional mutagenesis. Recent advances with the conceptually related *Flpe/FRT* system offers an alternative, and the two systems can be combined advantageously. The *cre/loxP* system requires the generation of two strains of mice. In one of them, the *Mof* sequence to be deleted upon recombination will be flanked by *loxP* sites (*Mof<sup>fllox</sup>*) introduced by homologous recombination in embryonic stem cells. The second mouse strain carries the *loxP* site-specific *cre* recombinase under control of a temporal- or tissue-specific promoter of choice.

(b) To determine whether ionizing radiation enhances neoplastic transformations in mouse embryonic fibroblasts of MOF heterozygous mice.

**e. Publications:**

We have achieved about 76% of envisaged goals for the second year of this grant. During the current funding period 20 papers were published and 2 are in press. Each paper

contributed to the over all goals of the proposal.

1. **Pandita T.K.** A multifaceted role for ATM in genome maintenance. *Expert Reviews in Molecular Medicine*. 5: 1-21 (2003).
2. **Pandita T.K.** and Roti Roti J.L. Role of Telomerase in Radiocurability. *Oncology Reports*. 10:263-270 (2003).
3. Sharma G.G, Gupta A., Scherthan H., Dhar S., Wang H., Gandhi V., Iliakis G., Young C.S.H., and **Pandita T.K.** hTERT associating with telomeres reduces spontaneous chromosome damage and enhances DNA repair. *Oncogene* 22:130-146 (2003).
4. Sarkar D, Leszczyniecka M, Kang DC, Lebedeva IV, Valerie K, Dhar S, **Pandita TK**, Fisher PB. Related Articles, Links Abstract Downregulation of Myc as a potential target for growth arrest induced by human polynucleotide phosphorylase (hPNPaseold-35) in human melanoma cells. *J Biol Chem*. 278:24542-24551 (2003).
5. Sharma G.G., Hall E.J., Dhar S., Gupta A, Rao P.H. and **Pandita T.K.** Telomere stability correlates with longevity of human beings exposed to ionizing radiations. *Oncology Reports* 10: 1733-1736 (2003).
6. Sharma GG, Hwang K-K., Pandita RK, Gupta A, Dhar S, Prentau M, Agarwal M, Worman HJ, Wellinger RJ, and **Pandita TK** (2003). Human heterochromatin protein 1 isoforms HP1 and HP1 interfere with hTERT-telomere interactions and correlates with changes in cell growth and response to ionizing radiation. *Mol Cell Biol* 23: 8363-8376.
7. **Pandita TK** (2004) Enrichment of cells in different phases of cell cycle by centrifugal elutriation. *Methods in Molecular Biology*. 241: 17-21.
8. **Pandita TK** (2004) Detecting influence of cell cycle regulatory proteins on human telomeres. *Methods in Molecular Biology*. 241: 329-339.
9. Hunt CR, Dix DJ, Sharma GG, Pandita RK, Gupta A, Funk M, and **Pandita TK** (2004) Genomic instability and enhanced radiosensitivity in Hsp70.1/3-deficient mice. *Mol Cell Biol* 24:899-911.
10. **Pandita TK**, Higashikubo R and Hunt CR. (2004) HSP70 and Genomic Stability. *Cell Cycle*. 3:591-592.
11. Richardson C, Horikoshi N and **Pandita TK** (2004) DNA double-strand break response network in meiosis. *DNA Repair* 3:1149-1164.
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13. Locke J, Zeug A, Thompson D, Allan J, Mazzarella K, Novak P, Hanson D, Singh A, Moros E and **Pandita TK** (2005) Localized versus regional hyperthermia: comparison of xenotransplants with a small animal ultrasound system and water bath limb immersion. *Int J Hyperthermia* 21: 271-281.
14. Puc J, Keniry M, Li HS, **Pandita TK**, Choudhury AD, Memeo L, Mansukhani M, Murty

- VVVS, Gaciong Z, Meek SEM, Piwnica-Worms H, Hibshoosh H and Parsons R (2005) Lack of PTEN sequesters CHK1 and initiates genetic instability. *Cancer Cell* 7:193-204.
15. Sarsour EH, Agarwal M, **Pandita TK**, Oberley LW, and Goswami PC (2005) Maganese superoxide dismutase protects the proliferative capacity of confluent normal human fibroblasts. *JBC* 280:18033-18041.
  16. Gupta A, Sharma GG, Young HCR, Agarwal M, Smith E.R., Paull TT, Lucchesi JC, Khanna KK, Ludwig T and **Pandita TK** (2005). Involvement of Human MOF in ATM Function. *Mol. Cell. Biol.* 25:5292-5305.
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  18. Ziv S, Brenner O, Amariglio N, Smorodinsky NI, Galron R, Carrion DV, Zhang W, Sharma GG, Pandita RK, Agarwal M, Elkon R, Katzin N, Bar-Am I, **Pandita TK**, Kucherlapati R, Rechavi G, Shiloh Y, and Barzilai A (2005). Impaired genomic stability and increased oxidative stress exacerbate different features of AT. *Hum Mol Genet* 14:2929-2943.
  19. **Pandita TK** (2005) Role of HSPs and telomerase in Radiotherapy. *Int Jr Hyperthermia* 21: 689-694..
  20. Pandita RK, Sharma GG, Laszlo A, Hopkins KM, Davey S, Chakhparonian M, Gupta A, Wellinger RJ, Zhang J, Powell SN, Roti Roti JL, Lieberman HB and **Pandita TK** (2006). Mammalian Rad9 plays a role in telomere stability, S- and G2-phase specific cell survival and homologous recombinational repair. *Mol Cell Biol.* 26:1850-1864.
  21. Bredemeyer AL, Sharma GG, Huang C-Y, Helmink BA, Nuskey B, Walker LM, **Pandita TK**, Bassing CH, and Sleckman BP (2006). ATM stabilizes DNA double strand break complexes during V(D)J recombination. *Nature* (2006) (in press).
  22. **Pandita TK** (2006). Role of mammalian Rad9 in genomic stability and ionizing radiation response. *Cell Cycle* (in press).

#### **f. Project-Generated Resources:**

Research supported by this grant resulted in generation of mouse heterozygous for MOF.

**Appendix: None**