# Chromatin Structure and Breast Cancer Radiosensitivity

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 radioresponsiveness and oncogenic transformation. If *hMOF* proves to be involved in the radioresponsiveness 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 RTPCR 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 during second year of funding:

During the second 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.

Task 2. (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. BgIII (B), EcoRI (E), XbaI (X), SalI (S).

Fig. 4. Southern analysis of tail DNA digested with BgIII 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).

we screened a genomic I 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 EcoRI 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+/-) 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.

➢ 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.

During the third 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 (Moflox) 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 70% of envisaged goals for the second year of this grant. During the current funding period 12 papers were published and 3 are submitted for publication. Each paper contributed to the over all goals of the proposal.


f. Project-Generated Resources:
Research supported by this grant resulted in generation of mouse heterozygous for MOF.

Appendix: None