Transactions of the Korean Nuclear Society Autumn Meeting
Gyeongju, Korea, November 2-3, 2006

Identification of Radiation Specific Gene Signatures in Rat Mammary Tumors

Yoon-Jin Lee[1,*], Hae-June Lee[1,*], Chang-Mo Kang[2], Sangwoo Bae[1], Dooil Jeoung[3], Ja-June Jang[4], Seung-Sook Lee[5] and Yun-Sil Lee[1,+]

[1]Laboratory of Radiation Effect, [2]Radiation Cytogenetics and Epidemiology and [4]Laboratory of Experimental Pathology, Korea Institute of Radiological and Medical Sciences, Seoul 139-706, Korea, [3]Division of Life Sciences, Kangwon National University College of Natural Sciences, Chuncheon 200-701, Korea, [4]Department of Pathology College of Medicine, Seoul National University, 110-108, Seoul

[*]Equally contributed

1. Introduction

It is well accepted that cancer arises in a multi-step fashion and that environmental exposures to physical and chemical agents are major etiological factors.

Exposure to carcinogens plays a major and probably in etiological role in the initiation of this human disease. In experimental animal models, ionizing radiation induces mammary carcinomas both *in vivo* and *in vitro*, however, the cellular and molecular mechanisms of radiation induced carcinogenesis are not known.

In this paper, we compare to gene expression patterns by cDNA microarray in gamma-radiation or DMBA induced rat mammary tumors and found that radiation specific gene expression signature was present in radiation induced breast cancer

2. Methods

Animals

Five-week old female Sprague-Dawley rats were purchased from SLC (Hamamatsu, Japan) and housed in autoclaved cages and maintained in a room with controlled temperature (22±1°C) and humidity (50± 5%) under a regular 14 hr light/10 hr dark cycle. All animals were maintained at the animal care facilities, and food and water were supplied *ad libitum*. Studies were conducted under guidelines for the use and care of laboratory animals and were approved by the Institutional Animal Care and Use Committee of the Korea Institute Radiological and Medical Sciences (KIRAMS)

Treatments

At 7 weeks of age, rats were divided into 2 groups: DMBA treated (n=24) and γ-irradiated (n=21). Rats were given whole-body γ-irradiation (1.5 Gy) (once a week, total 5 times) generated from a source ^{137}Cs source (Atomic Energy of Canada, Ltd., Ontario, Canada) with dose rate of 3.81 Gy/min. DMBA (15 mg/rat, Sigma) was treated by oral intubation, once. Palpable tumors were examined weekly and recorded. Rats were autopsied under ether anesthesia at 14 weeks of DMBA treatment or 26 weeks of last IR irradiation. Each palpable tumor was matched one by one to an excised tumor, and nonpalpable tumors were also removed. Collected tumors were fixed in 10% neutral buffered formalin, and paraffin-embedded sections were routinely prepared and stained with hematoxylin and eosin (H-E) for histologic evaluation. Parts of tumors >1cm in diameter, large enough for RNA isolation, were snap-frozen and stored at –80 °C.

3. Results

Mammary tumors were produced by radiation or dimethylbenz(a)anthracene (DMBA). Pathological analysis showed that radiation-induced mammary tumors were various stages of pathological phenotypes such as adenoma, papilloma, and carcinoma, however, in the case of DMBA-induced tumors, all were carcinoma. TUNEL positive cells were much more in radiation-induced tumors when compare to the DMBA induced tumors, while DNA damage response including p53 accumulation and histone H2AX phosphorylation was higher in DMBA induced tumors. cDNA microarray analysis using radiation- or carcinogene-induced tumor tissues which showed same pathological grade (carcinoma, grade I), revealed that 32 genes were specific for radiation-induced tumors and 11 genes were specific for DMBA induced tumors. Ten genes were induced in both radiation and DMAB induced tumors. Real time RT-PCR analysis to confirm, indicated that stanniocalcin, interferon regulatory factor, interleukin 18 binding protein, and chloride channel calcium activated 3 are expressed in both DMBA and radiation induced tumors, and arachidonate 5-lipoxygenase activating protein 1 (A5LAP) and cathepsin S were expressed in only radiation induced tumors. Soft agar growth assay were carried out to identify the cancer features of IR-specific genes. Expression of mRNA for A5LAP and cathepsin S was increased by radiation in MCF10A cells and the stable transfected cells of A5LAP and cathepsin S showed morphological changes compared to the control cells, such as the obvious pseudopods.

Table 1.Gene list which was confirmed by RT-PCR analysis

Acc no.	Title	Specificity
AI592910	Stanniocalcin 2	Up in both IR and DMBA
AI122010	Interleukin 18 binding protein	Up in both IR and DMBA
AI180945	Unknown	Up in IR and down in DMBA
AA596289	Chloride channel calcium activated 3	Up in both IR and DMBA
AA930477	Arachidonate 5-lipoxygenase activating protein	Up in IR
AI845967	Cathepsin S	Up in IR
AA939782	Interferon regulatory factor 1	Up in both IR and DMBA

4. Conclusion

These data suggest that the arachidonate 5-lipoxygenase activating protein 1 and cathepsin S may be related to cellular transformation induced by radiation. Our findings suggest that a possibility for radiation specific gene signature in radiation-induced mammary tumors.

REFERENCES

[1] Huang, L., Snyder, A.R. and Morgan, W.F. Radiation induced genomic instability and its implications for radiation carcinogenesis. Oncogene, 22, 5848-5854, 2003

[2] Boice, J.D., Land, C.E., Shore, R.E., Norman, J.E. and Tokunaga, M. Risk of breast cancer following low-dose exposure. Radiology, 131, 589-597, 1981

[3] Land, E.C., Boice, J.D. , Shore, R.E., Norman, J.E. and Tokunaga, M. Breast cancer risks from low-dose exposures to ionizing radiation; results of parallel analysis of 3 exposed populations of women. J. Natl. Cancer. Inst., 65, 353-376,1980

[4] Wazer, D.E., Chu, Q., Liu, X.L., Gao, Q., Safaii, H. and Band, V. Loss of p53 protein during radiation transformation of primary human mammary epithelial cells. Mol. Cell. Biol., 14, 2468-2478, 1994