

Short Communication

## Similarity of GATA-3 Expression between Rat and Human Mammary Glands

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**Abstract:** The GATA family members are zinc finger transcription factors involved in cell differentiation and proliferation. In particular, GATA-3 is necessary for mammary gland maturation and is a useful marker in the characterization of mammary carcinoma in humans. The expression of GATA-3 protein in normal mammary glands, fibroadenomas and carcinomas was immunohistochemically compared in female rats and humans. In normal mammary glands of rats and humans, scattered luminal cells in the acini and whole ductal epithelial cells were positive for GATA-3 in the nuclei. No positive cells were detected in rat or human fibroadenomas. In rat and human mammary carcinomas, the nuclei of proliferating luminal-derived cancer cells expressed GATA-3. Therefore, GATA-3 protein is a candidate marker for mammary carcinoma in rats as well as humans. (DOI: 10.1293/tox.2014-0008; J Toxicol Pathol 2014; 27: 159–162)

**Key words:** GATA-3, immunohistochemistry, mammary gland, mammary tumor, rats

Breast cancer is a leading cause of human mortality and morbidity worldwide<sup>1</sup> and is a subject of major research efforts utilizing rat models<sup>2</sup>. Fibroadenomas are the most common spontaneous tumors in female Sprague-Dawley (SD) rats, with incidences as high as 70% reported in long-term studies<sup>3</sup>. Rat mammary carcinoma, considered relevant to humans, has extremely high incidences of spontaneous development in chronic and carcinogenesis studies with SD rats<sup>3</sup>.

The GATA family, the members of which share a conserved zinc finger DNA-binding domain, is composed of GATA-1, 2, 3, 4, 5 and 6, and alterations in these transcription factors are causatively involved in various cancers in humans<sup>4</sup>. GATA-3 contributes to the development of multiple organ systems<sup>4</sup> and is essential for development of the mammary epithelium and is highly expressed, especially in normal luminal epithelial cells of humans and mice<sup>5–7</sup>. GATA-3 expresses several types of cancers in humans and has been reported as a sensitive and specific marker for the diagnosis of human breast carcinomas<sup>8–11</sup>. In one report, 91% of 99 ductal carcinomas and 100% of 48 lobular carcinomas were

positive<sup>8</sup>. In another report, 92% of 92 ductal carcinomas and 100% of 38 lobular carcinomas were positive<sup>9</sup>. GATA-3 is also expressed in male breast carcinomas but less often than in female carcinomas<sup>10</sup>. Therefore, it is recommended that GATA-3 be added to the routine initial screening panel for the diagnosis of breast carcinomas, especially metastatic carcinomas in serous effusion specimens<sup>11</sup>.

The molecular characterization of rat mammary tumors has been reported<sup>12, 13</sup>, however, no reports have compared *in vivo* data on GATA-3 expression between mammary tumors of rats and humans. The present study was conducted to compare the immunohistochemical characteristics of normal mammary glands, fibroadenomas, and carcinomas of rats and humans.

Mammary samples were fixed in 10% neutral buffered formalin, embedded in paraffin, sectioned at a thickness of 4  $\mu$ m and stained with hematoxylin and eosin (H&E). Histopathological terminology and diagnostic criteria for rodent mammary neoplastic lesions were in accordance with the International Harmonization of Nomenclature and Diagnostic Criteria for Lesions in Rats and Mice Project<sup>3</sup>. Human terminology and diagnostic criteria for mammary neoplastic lesions were in accordance with the guidelines of human tumor pathology<sup>14</sup>. Four human mammary fibroadenomas (patient ages: 39, 40, 52 and 55 years) and four carcinomas (patient ages: 57, 66, 73 and 78 years) were surgically removed at Kansai Medical University Takii Hospital during the past two years. Mammary carcinomas were invasive carcinomas of no special type. Normal tissues were obtained from four specimens from the patients with fibro-

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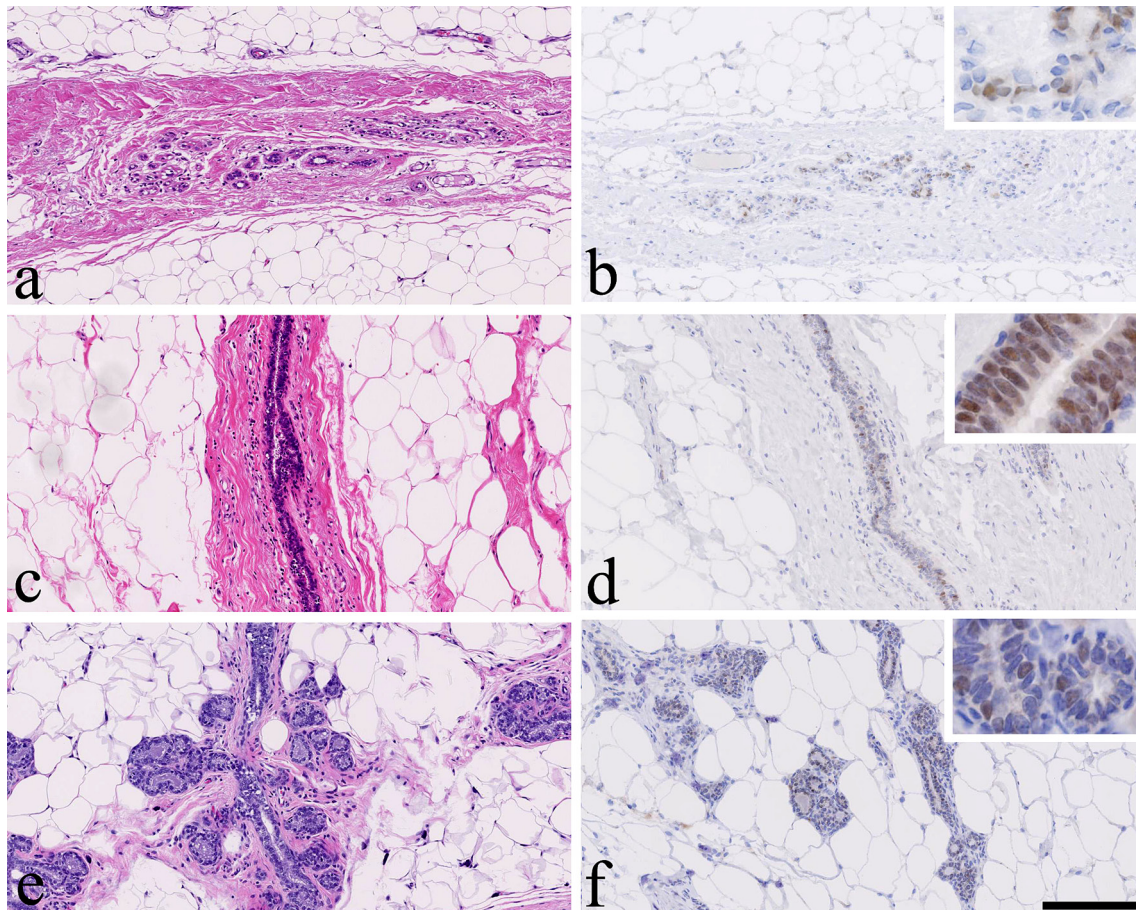
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adenomas. In rat cases, fibroadenomas were removed from seven SD rats without any treatment (Charles River Laboratories Japan, Yokohama, Japan) at the age of 78 weeks. The seven specimens of mammary carcinomas were derived from *N*-methyl-*N*-nitrosourea-exposed SD rats and harvested at the age of 20 weeks. Normal mammary tissues were obtained from saline-exposed SD rats at the age of 20 weeks that were used as controls. All mammary tissues and tumors were located in the inguinal area. All rats were anesthetized with isoflurane (Forane<sup>R</sup>; Abbott Japan, Tokyo, Japan) and sacrificed by exsanguination via aortic transection.

Sequential sections of normal mammary tissues and tumors were immunohistochemically evaluated with monoclonal antibody to GATA-3 (sc-268, 1:100 dilution; Santa Cruz Biotechnology, Santa Cruz, CA, USA). Sections were deparaffinized, hydrated and blocked for endogenous peroxidase. Heat-induced epitope retrieval was performed by using DAKO Target Retrieval Solution (pH 9.0; Dako, Carpinteria, CA, USA). The primary antibody was incubated overnight at 4 °C. The antigen-antibody complexes were

identified by using a streptavidin-biotin (LSAB) staining kit (Dako) according to the manufacturer's instructions. The reaction products were visualized with 3-3'-diaminobenzidine tetrahydrochloride. Nuclear staining of epithelial tumor cells for GATA-3 protein in at least 25% of mammary tumor cells or normal tissue cells was regarded as positive, according to the previously described method with some modifications<sup>8</sup>. Histopathological examination including immunohistochemical analysis was conducted by two toxicologic pathologists certified by the Japanese Society of Toxicologic Pathology (K.Y., A.T.) and by two human pathologists certified by the Japanese Society of Pathology (N.S., T.Y.).

Representative histology for normal mammary glands, fibroadenomas and carcinomas in the rat and human cases is shown in Fig. 1 and Fig. 2. Immunohistochemically, normal luminal epithelial cells in the acini and ducts of human mammary glands were positive for GATA-3 protein in all four cases (Fig. 1b, d). Positive reactions occurred in the nuclei but not in the cytoplasm. No myoepithelial cells



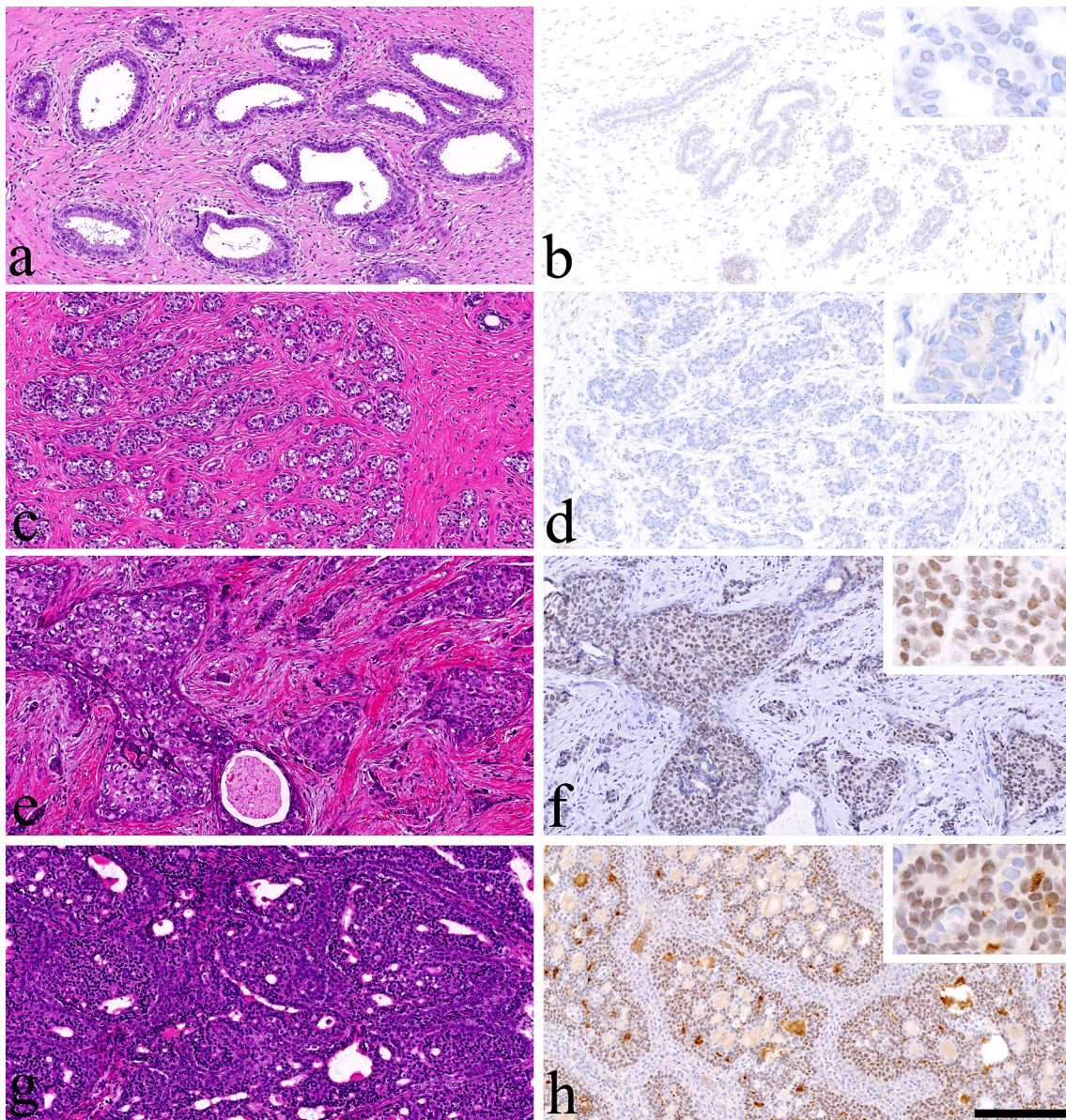
**Fig. 1.** Normal mammary glands. (a) Human acinar structure. H&E. (b) GATA-3 immunohistochemistry for the human acinar structure. Note the scattered nuclear positivity of acinar cells (insert). (c) Human mammary duct. H&E. (d) GATA-3 immunohistochemistry for the human mammary duct. Note the diffuse positivity in the nuclei of the ductal epithelium (insert). (e) Rat mammary acinus and duct. H&E. (f) GATA-3 immunohistochemistry for the rat acinar and ductal epithelial cells. Note the nuclear positivity of the acinar cells (insert) and ductal epithelial cells. Bar = 200  $\mu$ m.



of mammary glandular trees or stromal cells showed any positive reactions. There was a similar tendency for GATA-3 staining in the normal mammary glands of seven rats; scattered luminal cells in the acini and whole luminal cells in the ducts were positive for GATA-3 protein (Fig. 1f). In all rat and human fibroadenomas, no GATA-3 signals were seen in the epithelial and stromal components (Fig. 2b, d). In contrast, human mammary carcinoma cells from invasive carcinomas of no specific type showing no glandular differentiation (grade III) revealed diffuse nuclear positiv-

ity for GATA-3 protein (Fig. 2f). Likewise, in rat mammary carcinomas, GATA-3 protein was expressed in the nuclei of proliferating luminal-derived cancer cells, leaving basal (myoepithelial) cells negative. The positivity also revealed secretory contents produced by carcinoma cells. Mammary carcinomas in the rat and human cases examined possessed similar positivity in proliferating luminal-derived cells. In our human cases, there was a lack of myoepithelial differentiation.

The present study compared the immunohistochemi-



**Fig. 2.** Immunohistochemistry for mammary fibroadenoma and carcinoma. (a) Human fibroadenoma. Irregular ductal structures within the fibrous stroma are seen. (b) GATA-3 immunohistochemistry for human fibroadenoma. Note the negative reaction in tumor cells (insert). (c) Rat fibroadenoma (d) GATA-3 immunohistochemistry for rat fibroadenoma. Note the negative reaction in tumor cells (insert). (e) Human invasive mammary carcinoma tumor cells are seen. (f) GATA-3 immunohistochemistry for human mammary carcinoma. Note the diffuse nuclear positivity of cancer cells (insert). (g) Rat mammary carcinoma. (h) GATA-3 immunohistochemistry for rat mammary carcinoma. Note the diffuse nuclear positivity of cancer cells (insert) and secretory components. Bar = 200  $\mu$ m.



cal characteristics of normal mammary glands, fibroadenomas and carcinomas of rats and humans. GATA-3 protein was expressed in acinar and ductal epithelia of rats and humans, in agreement with a previous human report<sup>5</sup>; in normal mammary glands, GATA-3 is the most highly enriched transcription factor in both the terminal end buds (13-fold change) and the mature duct (10.6-fold change) microenvironment according to microarray data<sup>5</sup>. As in the human cases, GATA-3 protein was expressed in rat mammary carcinomas in the present study. Low GATA-3 expression has also been suggested to correlate with poor prognosis in human mammary cancers, and higher expression predicts better survival in patients with breast cancer<sup>15</sup>. GATA-3 drives human invasive mammary cancer cells to undergo reversal of the epithelial-mesenchymal transition, leading to the suppression of cancer metastasis<sup>16</sup>. In the present study, no GATA-3-positive cells were detected in rat and human fibroadenomas. In human benign breast lesions from fine-needle aspiration biopsy specimens, 64% of cases were negative for GATA-3 antibody immunohistochemically<sup>8</sup>. Further studies are necessary to understand this discrepancy in GATA-3 expression in benign mammary tumors.

Toxicologic pathologists do not generally use immunohistochemical markers for mammary glands in two-year carcinogenesis studies. However, good markers for tumor diagnosis may be very useful in risk assessment if treatment-related changes are observed. Additional immunohistochemical analyses are needed to understand the biology of chemically induced and spontaneously occurring mammary tumors in rats; however, the present research might provide valuable information on rat mammary carcinomas; GATA-3 may be a useful marker for malignant mammary tumors in rats as in human cases.

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