ONCOLOGY

GATA3 expression in estrogen receptor α-negative endometrial carcinomas identifies aggressive tumors with high proliferation and poor patient survival

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OBJECTIVE: The transcription factor GATA3 has recently been found to be involved in the carcinogenesis for numerous cancers. We investigated this marker in relation to clinicopathologic characteristics, hormone receptors, other biomarkers, and survival in endometrial carcinoma.

STUDY DESIGN: A population-based study of 316 endometrial carcinomas with complete follow-up was studied for GATA3, estrogen receptor (ER)-α, ERβ2, and progesterone receptor (PR) expression.

RESULTS: Positive GATA3 expression in hysterectomy specimens significantly correlated to high International Federation of Gynecology and Obstetrics stage, serous papillary/clear cell subtypes, high histologic grade, loss of PR expression, aneuploidy, high proliferation, pathologic p53 and p16 expression, and poor prognosis ($P = .003$). Loss of hormone receptors significantly correlated with aggressive phenotype and poor prognosis. Pathologic expression of GATA3/ERα in combination added independent prognostic information.

CONCLUSION: GATA3 expression is associated with an aggressive phenotype and adds independent prognostic information in addition to receptor status. Further studies of its value in tailored treatment protocols seem justified.

Key words: endometrial carcinoma, estrogen receptor-β2(cx), GATA3, prognostic markers, steroid receptors, survival

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Endometrial cancer is 1 of the most common malignant tumors of the female genital tract and is increasing in developed countries. Type I endometrial cancer, accounting for around 80% of the cases, is usually of the endometrioid type, well differentiated, and associated with hyperestrogenic risk factors like diabetes and obesity. Type II is more often of the nonendometrioid type, poorly differentiated, and not associated with estrogenic risk factors.[1,2]

Transcription factors are proteins that bind to promoters and thereby regulate gene transcription, and growth hormones mediate diverse actions by influencing transcription. Multiprotein complexes involving transcription factors and core regulators, and additional nuclear proteins are formed and serve as targets for regulation by hormones and signaling pathways.[3] Transcription factors are organized into structurally similar multigene families (*GATA* and *FOX*) and these genes play essential roles in activating or repressing target genes by chromatin remodeling and deoxyribonucleic acid (DNA) methylation.[4]

Several studies suggest a role of the transcription factor GATA3 in different human cancers because it is known to regulate the lineage determination and differentiation of many cell types such as in breast, lung, and prostate.[5-8] GATA3 is associated with estrogen receptor (ER)-α expression in breast cancer and is believed to be involved in development and differentiation of luminal cells.[6,8-10]

The correlation between steroid receptors (ER and progesterone receptor [PR]) and known prognostic variables such as tumor stage (International Federation of Gynecology and Obstetrics [FIGO]), histologic grade, depth of myometrial infiltration,and survival has been well documented in endometrial carcinomas.[11] Because most of these tumors are estrogen related, there is a strong focus on the molecular mechanisms of estrogen and the estrogen-related cofactors in relation to therapeutic strategies. Recent genetic, biochemical, and pharmacological dissection of the estrogen signal transduction pathway has led to the identification of numerous proteins and processes that have an impact on ER function. The association between ER and GATA3 expression in breast cancer indicates an important functional role for GATA3 in hormone-responsive cancers. It has been suggested that GATA3 is involved in regulating ER expression and that GATA3 might be predictive of hormone response.[10]

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The expression pattern and significance of GATA3 has to the best of our knowledge not been studied in endometrial carcinomas previously. On this background, we have examined the expression of ER isoforms and PR in relation to GATA3 staining in a population-based series of endometrial carcinomas with complete follow-up. Furthermore, we studied whether these markers can be used preoperatively as prognostic tools in identifying high-risk patients prior to primary surgical treatment. In particular, we examined whether GATA3 expression could be used as a prognostic supplement to the established steroid factors in endometrial carcinoma.

MATERIALS AND METHODS

All 316 patients diagnosed with endometrial carcinoma in a 10 year period (1981-1990) in Hordaland County, Norway, were studied retrospectively.[12] Several clinicopathologic variables were recorded: age at the time of diagnosis, FIGO stage according to the 1988 criteria,[13] revised histologic type and grade, treatment and survival. Data from the tumor markers (DNA index, vessel infiltration, proliferation [mitotic count, Ki-67 expression], and p53 and p16 expression in tumor tissue) was available from previous studies for comparisons.[14,15] Microscopic slides were reclassified and graded by 2 pathologists (I.M.S., L.A.A.) according to the 1994 World Health Organization criteria.[16]

Of the 316 patients diagnosed with endometrial carcinoma in the study period, 12 were excluded because of changed diagnosis at reclassification and 5 because of a diagnosis based on cytological examination only with no histologic material available.[14] Of the remaining 299 cases, paraffin blocks from the primary tumor were available in 286 cases (96%) and from the curettage specimens in 238 cases (80%). The treatment protocol and follow-up data have been described in detail in previous publications.[12,15]

Tissue microarray (TMA)

The TMA technique has previously been described and validated in several studies.[17,18] TMA constructions were made by identifying the area of highest tumor grade on hematoxylin and eosin–stained slides, followed by punching out 3 tissue cylinders with a diameter of 0.6 mm from the selected areas of the donor block and mounted into a recipient paraffin block using a custom-made precision instrument (Beecher Instruments, Silver Spring, MD). Sections of the resulting TMA blocks (5 um) were made by standard technique. TMA blocks were made from curettage material and corresponding tumor tissue from hysterectomy specimens.

Immunohistochemistry

TMA slides were dewaxed with xylene/ethanol before microwave epitope retrieval boiling for 10 minutes at 750 W and 20 minutes at 350 W in tris (hydroxymethyl) aminomethane-EDTA buffer (pH 9). For ERα and PR, the staining procedures were performed on the Dako Cytomation Autostainer (Copenhagen, Denmark), blocking the slides with peroxidase (S-2023) for 5 minutes, and then the sections were incubated for 30 minutes with the monoclonal antibodies ER50 (M 7047) diluted 1:50 and PR 636 (3569) diluted 1:150 (Dako, Copenhagen, Denmark). The ERβ2 isomer was incubated for 1 hour with ab27961 diluted 1:50 (Abcam, Cambridge, UK). Regarding GATA3, staining was performed manually and the sections were incubated overnight at 4°C with monoclonal sc-268 diluted 1:10 (Santa Cruz Biotechnology, Santa Cruz, CA). Immunoperoxidase staining was carried out using the En Vision chain-polymer method (Dako Cytomation) with diaminobezidine peroxidase and counterstained with Harris hematoxylin.

Evaluation of staining

Blinded for patient characteristics and outcome, the slides were evaluated in a standard light microscope for immunohistochemical staining by 2 of the authors (I.B.E., H.B.S.). For all markers we used a semiquantitative and subjective grading system considering both the intensity of staining and the proportion of tumor cells in the tissue section showing an unequivocal positive reaction in the cell nuclei.[14] Staining intensity was graded from 0 (no staining) to 3 (strong staining). The percentage of immunopositive cells was graded as 0 (no tumor cells positive), 1 (positive staining in less than 10% of the tumor cells), 2 (positive staining in 10-50% of the tumor cells), or 3 (positive staining in greater than 50% of the tumor cells). A staining index (SI) was calculated as the product of staining intensity and staining area (values 0-9).

In the statistical analyses, cutoff values for SI were based on median or quartile values, considering the frequency distribution for each marker as well as the size of subgroups and number of events in each category in survival analysis.

Kappa values for intra- and interobserver reproducibility were estimated and found to be acceptable for all markers studied (k greater than 0.75).

Statistical analysis

Analyses were performed by the statistical software package SPSS 11.0 (SPSS Inc, Chicago, IL). Associations between different categorical variables were assessed by Pearson's χ^2 test. Univariate analyses of time to death caused by endometrial carcinoma were performed using the Kaplan-Meier method. Differences in survival between categories were estimated by the log- rank (Mantel Cox) test. The variables with significant impact on survival in univariate analyses ($P \leq .05$) were further examined by log-minus-log plot to decide how these variables could be incorporated in the Cox' proportional hazard regression model. Unadjusted and adjusted hazard ratios were estimated as a measure of effect.

RESULTS

GATA 3 expression

Distinct nuclear staining for GATA3 was seen in 23% of the hysterectomy specimens and was significantly correlated to high FIGO stage, serous papillary/clear cell subtypes, high histologic grade, aneuploidy, and a panel of other biomarkers (Table 1). No nuclear staining was seen in benign endometrium specimens used as controls. Analyzing the subgroup of endometrioid tumors only, we found a similar pattern with highly significant associations between positive nuclear GATA3 staining and high FIGO stage ($P = .02$); high histologic grade ($P = .02$);