Androgen effects in ovarian epithelial cells potentially involved in cancer predisposition or progression

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ABSTRACT

The main objective of this thesis is to further explore the role of androgen in initiation/progression of ovarian cancer and to uncover the underlying mechanism (s). Based on previous experiments in our lab, some androgen responses were dysregulated in malignant ovarian epithelial cells (OVCAS) as well as in ovarian surface epithelial cells (OSE) derived from patients with germline *BRCA-1* or *-2* mutations (OSEb) relative to non-malignant ovarian surface epithelial (OSE) cells. Potentially, such altered responses may be involved in ovarian carcinogenesis/progression and so gene expression profiling by cDNA microarrays was initiated to explore the global effect of androgen on gene expression profiles of these cultures. The results demonstrate that ovarian epithelial cells respond to androgen by up-regulating hundreds of genes, from which a subgroup was verified by real-time RT-PCR. The analysis of microarray data was combined with the information derived from known/predicted protein interactions in the form of networks defining the pathways of cellular functions to generate hypotheses on potential downstream effects of androgen on individual targets. Potentially, disruption of protein-protein interactions in critical pathways may lead to cancer. Since previous work had indicated that androgen blocks the growth suppressing actions of TGF-β on ovarian epithelial cells and due to the density of the overall interaction network, I chose to focus on the TGF-β neighborhood. In the microarray studies, FLJ12604 was upregulated in OVCAS due to androgen treatment, further verified by real-time RT-PCR. This gene was also upregulated in OSE and OSEb due to dihydrotestosterone (DHT) treatment. Furthermore, based on Lumier experimental results, FLJ12604 interacts with transforming growth factor receptor I (TBRI). Therefore, it was hypothesized that the androgen inhibitory effect on TGF-β inhibition in ovarian cancer may be mediated by this gene, potentially causing initiation/progression of the cancer. To test this, a transfection experiment was designed in OVCAS cultures to downregulate FLJ12604 by SiRNA. If indeed FLJ12604 acts to inhibit TGF-β signaling, through its interaction with TBRII, then a decrease in TGF-β-induced luciferase levels in cells overexpressing this protein is expected. If androgen increases endogenous FLJ12604 in these cells, then androgen treatment should result in a decrease in TGF-β-induced luciferase expression and treatment with siRNA for FLJ12604 should block this. However, due to the primary nature of the OVCAS culture, the experiment was not completed and remains to be further continued. Furthermore, a total of 25 androgen-altered genes were differentially expressed in OSEb and OVCAS as compared to OSE, a subset of which was verified by real-time RT-PCR. Out of these genes, 9 mapped to the protein-protein interaction database, and were networked within two interacting partners. Basic leucine zipper transcription factor 2 (BACH2) and acetylcholinesterase (ACHE), which were up-regulated by androgen in OSEb relative to OSE, were further investigated using an ovarian cancer tissue microarray from a separate set of 150 clinical samples. High levels of cytoplasmic ACHE staining correlated with decreased survival, whereas nuclear BACH2 staining correlated with decreased time to disease recurrence. This finding supports a potential role for altered androgen effects in ovarian cancer initiation/progression. In addition to BACH2 and ACHE, this study highlights a set of potentially functionally related genes for further investigation in ovarian cancer. However, two questions were raised from microarray study: 1) the possibility of existence of differences in gene expression in OSE and OSEb cultures in the absence of DHT and 2) the possibility of existence of differences between OSE from *BRCA1* and *BRCA2* mutation carriers. To address these, an independent set of samples were derived and treated as in the previous microarray experiment. RNA was extracted and applied to the Hu133 plus 2.0 Affymetrix chips. Based on the analysis of the Affymetrix data obtained, differences exist in gene expression profiles from OSE and OSEb with the greatest difference observed with mutated *BRCA2*. Additional cultures are likely required to reliably detect androgen altered gene expression in paired analyses, but the data obtained from this experiment, suggest a muted response to androgen in *BRCA2* mutation carriers.