学 位 論 文 の 要 旨

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学 位 論 文 名　Fatty Acid Synthase Expression Associated With NAC1 Is a Potential Therapeutic Target in Ovarian Clear Cell Carcinomas

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論 文 内 容 の 要 旨

INTRODUCTION

Ovarian clear cell carcinoma (OCCC), is one of four major histological types (serous, mucinous, endometrioid, and clear cell), constitutes around 25% of all ovarian carcinoma in Japan, carries a poor prognosis despite 60% being diagnosed at an early stage. Unlike its serous counterpart, OCCC is more frequently platinum resistant. NAC1 is a member of the BTB/POZ family of proteins that participate in several cellular functions including proliferation, apoptosis, transcription control, and cell morphology maintenance. NAC1 is significantly overexpressed in several types of human carcinomas and have a role in taxane resistance. Using a quantitative proteomic method employing tandem mass spectrometry (MS/MS) and spectral counting, Ueda et al demonstrated that NAC1 regulates Fatty Acid Synthase (FASN) expression. FASN, the enzyme responsible for the *de novo* synthesis of fatty acids, has emerged as a potential therapeutic target in human cancers. The upregulation of FASN expression in cancer cells has been linked to both MAPK and PI3K pathways through the sterol regulatory element binding protein 1c. FASN expression denotes poor prognosis in breast and prostate cancers. In this study, we further investigated the clinicopathological role of NAC1-regulated FASN expression in OCCC, as well as the possibility of FASN-based therapeutics in OCCC.

MATERIALS AND METHODS

Formalin-fixed, paraffin-embedded 144 ovarian cancers samples (44 serous, 10 mucinous, 60 clear cell and 20 endometrioid) organized into tissue microarrays were used. Immunohistochemistry was performed on tissue microarrays after deparaffinisation and incubated with anti-FASN and anti-NAC1 antibody followed by scoring according to immune

intensity from 0 to 3. Fluorescence *in situ* hybridization was done using BAC clones RP11-356L15 and CTD-2508D10 containing the genomic sequences of the 19p13.2 amplicon for NAC1 target probes and CTD-2518O18 for reference probe generation. Western blot analysis was done on OCCC cell lines (ES2, JHOC9, JHOC5, OVISE, OVTOKO, RMG1, OV207) and probed with NAC1(1:100) and FASN(1:1000) antibody. GAPDH was used for loading controls. Knockdown of gene expression was done with two siRNAs targeting NAC1 and luciferase siRNA was used as control. Following siRNA transfection, quantitative real-time RT-PCR analysis of NAC1 and FASN mRNA expression level was also done. MTT growth assay was done after 96 hrs of treatment with 10mM of C75(FASN inhibitor). The pCMV/NAC1 vector was stably transfected into the ES2 cell line using the Nucleofector II electroporator and stable transfected cells were in section media containing 3-6 μg/ml blasticidine. Statistical methods for clinical correlation progression-free and overall survivals were calculated from the date of diagnosis to the date of first relapse or last follow-up. The data were plotted as Kaplan–Meier curves, and the statistical significance was determined by the log-rank test. Data were censored when patients were lost to follow-up. The Student's t-test (comparison of two groups) or ANOVA (for comparison of more than two groups) were used to evaluate numerical data.

RESULTS AND DISCUSSION

High expression of NAC1 (immunointensity 2+ and 3+) was observed in 33.3% (44/132) of all analyzed tumors including 15/43 (35%) serous, 1/11 (9%) mucinous, 4/18 (22%) endometrioid, and 24/60 (40%) of clear cell carcinomas. Interestingly, the frequency of high NAC1 protein expression was highest in OCCC and accordingly we focused on OCCC for further investigations. *NACC1* gene amplification is a rare event in OCCC as we also found no amplification within 58 (0%, 0/58) informative samples. On the other hand, high NAC1 expression is significantly correlated with high FASN expression status (p=0.018). Kaplan–Meier survival analysis showed high expression of FASN expression correlates significantly with shorter progression-free survival of OCCC patients treated with platinum-based chemotherapy (p=0.021). Interestingly, OCCC patients with high NAC1 expression or high FASN expression had significantly shorter progression-free and overall survival compared with patients with low expression of these genes (p=0.004 and 0.037) respectively. Moreover, western blot analysis in OCCC cell lines showed, NAC1 protein expression levels significantly correlated with FASN protein expression level (p<0.01). *NAC1* knockdown leads to decreased FASN expression in OCCC cell lines. Constitutive expression of NAC1 also leads to increased FASN expression as stably transfected with a NAC1 pCMV vector ES2 cell line showed higher *FASN* gene expression levels as measured by real-time PCR. To assess the contribution of FASN expression to cell growth and survival, OCCC cell lines were treated with the FASN inhibitor C75. The FASN protein expression status tended to be correlated with growth inhibition by the C75 in these cell lines.

Ovarian clear cell carcinomas are more aggressive and carry a worse prognosis than stage-matched serous adenocarcinomas, likely because OCCC is frequently refractory to platinum-based chemotherapy. The current immunohistochemical analysis demonstrated strong expression of NAC1 in 40% of clear cell carcinomas, in comparison with 9–35% of serous,

endometrioid and mucinous carcinomas. *NACC1* gene amplification accounts for the increased expression in many high-grade ovarian serous carcinomas; however, some serous carcinomas did have increased NAC1 expression in the absence of gene amplification. In contrast, *NACC1* gene amplification was undetectable in all clear cell carcinoma specimens tested, which suggests that NAC1 in this histology may be regulated at the transcriptional level. Recently, Ueda et al reported that FASN is a potential downstream target of NAC1 in serous high-grade ovarian carcinoma. Therefore, to assess the relationship between NAC1 and FASN we first knocked down NAC1 in OCCC lines, JHOC9 and OV207, using siRNA. Reduction of NAC1 expression resulted in decreased FASN expression in the NAC1-knockdown cell line, indicating that FASN was a likely downstream target of NAC1. Conversely, an ES2 cell line overexpressing NAC1 had significantly increased *FASN* gene expression. These reciprocal findings suggest that FASN is a potential downstream target of NAC1 in OCCCs. FASN overexpression were more sensitive to a potent FASN inhibitor, C75, suggesting that FASN-targeted therapy may have activity in this subset of OCCC. The mechanism underlying the upregulation of FASN in OCCC is not clear and likely involves multiple pathways. FASN overexpression also increases EGFR and HER2 protein expression and tyrosine phosphorylation and thereby amplifies oncogenic signaling pathways that contribute to tumorigenic transformation. In OCCC, the NAC1 pathway represents another mechanism for controlling FASN expression and pathway activity. Unlike other members of the BTB/POZ family, NAC1 has a BEN domain instead of the zinc-finger DNA-binding domain. It does act as a transcription corepressor with other BTB/POZ proteins. Further studies are required to elucidate the transcriptional regulation of *FASN* by NAC1. As with carcinomas of the colon, prostate and breast, FASN overexpression appears to correlate with worse prognosis and found more frequently in the aggressive OCCC. Moreover, it has also been shown to interact with nuclear proteins potentially involved in tumorigenesis, including Nanog, CoREST, HDAC3 and HDAC4. Thus, it is possible that FASN expression is indirectly controlled by NAC1 through binding with its specific partner(s). More importantly, in patients with OCCC, the high level of FASN significantly correlated with poor prognosis, suggesting that FASN contributed to the aggressive phenotype. How FASN contributes to disease aggressiveness in OCCC remains to be elucidated. Besides endowing drug resistance, FASN may enhance oncogenesis via the Wnt, c-Met, and proteosome pathways. Moreover, upregulation of FASN confers a growth and survival advantage by blocking apoptosis under hypoxia, a common condition in solid tumors and malignant effusions. Identification of the NAC1/FASN pathway sheds new light on the molecular mechanism by which NAC1 promotes tumor progression in OCCC.

CONCLUSIONS

In conclusion, we demonstrated that NAC1 and its potential downstream target FASN are overexpressed in a subset of OCCC. Furthermore, NAC1/FASN expression is a biomarker of poor outcome for patients treated with conventional platinum-based chemotherapy in OCCCs. New-generation FASN inhibitors deserve consideration in future clinical trials involving OCCC, particularly for patients who are refractory to platinum-based chemotherapy.