The role of 14-3-3 proteins in gynecological tumors

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1. ABSTRACT

A large family of highly conserved cellular 14-3-3 proteins plays key roles in the regulation of central physiological pathways such as metabolism, protein trafficking, signal transduction, apoptosis and regulation of cell cycle. The involvement of these proteins in the regulation of various tumor suppressor genes and oncogenes points to their potential role in human cancer. According to some, the 14-3-3 proteins in gynecological tumors, promote gynecological tumors whereas others suggest that 14-3-3 proteins function as tumor suppressors in such tumors. Here, we review the use of 14-3-3 proteins as novel markers for screening and early diagnosis of gynecological malignancies, and in monitoring the effectiveness of treatment. 14-3-3 proteins are proposed to be used as prognostic factors and as specific target in the treatment of cancers.

2. INTRODUCTION

The 14-3-3 proteins are a large family of small, highly conserved, widely expressed 28–33-kDa acidic polypeptides that are found in all eukaryotic species (1–2). In humans, seven different genes encode the highly conserved 14-3-3 isoforms beta, gamma, epsilon, eta, sigma, tau, and zeta. Most of the isoforms are expressed in all tissues, although 14-3-3 sigma expression is restricted to epithelial cells (3). The 14-3-3 proteins interact physically with a wide variety of other cellular proteins through a conserved amino acid motif, typically composed of a phosphorylated serine or threonine flanked by an arginine and a proline (4–5). It is through their binding to their target proteins that the 14-3-3 proteins function as adapters, regulators, and chaperones, allowing them to participate in a wide variety of cellular processes, including metabolism, signal transduction, protein trafficking, programmed cell death, and cell-cycle regulation (6–7).

There is mounting evidence that the 14-3-3 proteins play roles in human tumorigenesis. Several studies have reported increased 14-3-3 protein expression in specific cancers, including the increased
expression of 14-3-3 zeta in lung cancers, oral squamous cell carcinomas, stomach cancers, and papillomavirus-induced carcinomas; increased 14-3-3 beta, gamma, and tau expression in lung cancer biopsies; and increased 14-3-3 gamma expression in chemoresistant melanomas (8). Several studies have reported that the overexpression of 14-3-3 sigma might predict poor prognoses in colorectal, prostate, and pancreatic cancers, and might contribute to tumor resistance to DNA-damaging drugs in some tumors, suggesting that the 14-3-3 proteins promote tumorigenesis and tumor progression. However, 14-3-3 sigma has been considered a tumor suppressor in many cancers for many years, and the downregulation of 14-3-3 sigma has been observed in numerous cancers of epithelial origin, including lung carcinomas (9). Thus, the roles of the 14-3-3 proteins in tumor development are complex and far from clear. The possible contribution of the 14-3-3 proteins to tumorigenesis has attracted the increasing attention of basic and clinical researchers.

To date, the data have been conflicting, describing a supportive or a possibly inhibitory role for the 14-3-3 proteins in gynecological tumors (10–12). Therefore, in this review, we discuss the results of recent publications, which suggest the use of the 14-3-3 proteins as novel markers of gynecological tumors for tumor screening, early diagnosis, and treatment monitoring. The possible utilization of the 14-3-3 proteins as prognostic factors and specific therapeutic targets is also reviewed.

3. ROLES OF 14-3-3 PROTEINS IN CERVICAL CANCER

3.1. Changes in 14-3-3 proteins in cervical carcinogenesis

In a comparison of the gene expression profiles of six cervical cancer cell lines (HeLa, CaLo, SiHa, CaSki, ViBo, and C33A) and the nontumorigenic cell line HaCaT, Higareda-Almaraz et al. (13) reported high levels of 14-3-3 zeta protein in all the cervical cancer cell lines but not in the control cells. They also confirmed that in cervical cancer cell lines, 14-3-3 zeta is the ultimate determinant of cell fate, causing cell-cycle deregulation and the consequent malignant transformation of the cell. Recently, Boon and Banks (14) confirmed that high-risk human papillomavirus (HPV) E6 oncoproteins directly and specifically interact with 14-3-3 zeta, although there were significant differences in the efficiencies with which the HPV-16, HPV-18, and HPV-31 E6 oncoproteins associated with 14-3-3 zeta, suggesting that 14-3-3 zeta is associated with the development of cervical cancer.

In a study of cervical biopsy specimens from 225 women diagnosed with HPV infection or cervical intraepithelial neoplasia (CIN), Syrjänen et al. (15) demonstrated that the expression of 14-3-3 sigma increased in parallel with the grade of CIN lesions. The upregulated expression of 14-3-3 sigma was also significantly related to the detection of high-risk HPV, including types 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, and 68, and showed a linear relationship with high-risk HPV loads. 14-3-3 sigma expression was of no value in predicting the outcomes (incidence, persistence, or clearance) of high-risk HPV infections or incident CIN 1+ and CIN 2+. 14-3-3 sigma was not inactivated in cervical carcinoma or CIN, but was upregulated on during the transition from CIN 2 to CIN 3. Therefore, the upregulation of 14-3-3 sigma expression is associated with high-grade CIN and high-risk HPV at baseline.

Holm et al. (10) reported high cytoplasmic 14-3-3 sigma protein expression in all 10 cases of normal cervical epithelium, with no nuclear staining. However, 14-3-3 sigma protein was reduced in the cytoplasm and shuttled to the nucleus in a relatively large number of cervical squamous cell carcinomas, suggesting that 14-3-3 sigma acts in the carcinogenesis of cervical squamous cell carcinoma via two different mechanisms: a reduction in 14-3-3 sigma protein and its nuclear translocation. However, there was no association between 14-3-3 sigma mRNA levels and its protein expression, regardless of whether the protein level in the cytoplasm, nucleus, or both was considered. This supports the posttranscriptional regulation of 14-3-3 sigma in cervical squamous cell carcinoma.

3.2. Roles of 14-3-3 proteins in the treatment of cervical cancer

The activities of various natural dietary agents, especially spices and herbs, in health promotion, including the suppression of cancer, have attracted considerable attention (16). Liu et al. (17) reported that 6-shogaol, a natural compound isolated from the rhizome of ginger, induced apoptosis and G2/M-phase cell-cycle arrest in human cervical cancer (HeLa) cells. In a proteomic analysis, they found that the peak intensities of 76 proteins in HeLa cells changed (54 upregulated and 22 downregulated) by more than two-fold after treatment for 24 h with 15 μM 6-shogaol, relative to their levels in vehicle-treated cells. An Ingenuity Pathway Analysis of these proteins showed that 11, including four isoforms of the 14-3-3 proteins, were involved in networks that might be significantly associated with the anticancer mechanism of 6-shogaol.

Yue et al. (18) showed that ganoderic acid D, one of the major components of the Ganoderma triterpenes, inhibited the proliferation of HeLa cells and induced G2/M cell-cycle arrest and apoptosis. To identify the cellular targets of ganoderic acid D, they used with an in silico drug-target-searching program to predict that the six members of the 14-3-3 family (14-3-3 zeta/delta, 14-3-3 beta/alpha, 14-3-3 sigma, 14-3-3 theta, 14-3-3 gamma, and 14-3-3 epsilon) bind directly to ganoderic...
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Acid D. These results suggest that the 14-3-3 proteins play important roles in the cytotoxicity mechanism of ganoderic acid D (Figure 1). Using two-dimensional electrophoresis and matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) mass spectrometry, Yue et al. (19) then demonstrated that 14-3-3 beta/alpha protein was upregulated in HeLa cells after treatment with five purified ganoderic acids (ganoderic acids F, K, B, D, and AM1), which suggests that 14-3-3 beta contributes to the cytotoxicity of ganoderic acid and might be a target protein of the Ganoderma triterpene.

3.3. Roles of 14-3-3 proteins in the prognosis of cervical cancer

Holm et al. (10) investigated the expression of 14-3-3 sigma in a series of 297 cervical squamous cell carcinomas to clarify its prognostic value. They demonstrated with a univariate analysis that increasing age and the International Federation of Gynecology and Obstetrics (FIGO) stage correlated significantly with shorter disease-specific survival and disease-free survival, whereas the expression of 14-3-3 sigma in the cytoplasm, nucleus, or cytoplasm/nucleus was not significantly related to disease-specific survival or disease-free survival. This suggests that the 14-3-3 sigma protein has no prognostic value in cervical squamous cell carcinoma.

4. ROLES OF 14-3-3 PROTEINS IN BREAST CANCER

4.1. Changes in 14-3-3 proteins in breast carcinogenesis

4.1.1. Changes in 14-3-3 sigma in breast carcinogenesis

Moreira et al. (20) reported that the level of 14-3-3 sigma expression in malignant breast epithelial tissue was similar to that in matched nonmalignant tissue, with only sporadic loss of expression observed in three of the 68 tumors examined. These data suggest that the loss of 14-3-3 sigma expression is not a frequent event in breast carcinogenesis.

In contrast, using a serial analysis of gene expression, Ferguson et al. (11) demonstrated that the expression of the 14-3-3 sigma gene was seven-fold lower in three breast carcinoma cell lines (21PT, 21MT, and MDA-MB-468) than in two normal human mammary epithelial cells (MCF-10A and HBL-100). This finding was verified with northern blotting, which showed that 14-3-3 sigma mRNA was undetectable in 45 of 48 primary breast carcinomas, whereas it was readily detected in all six finite-life-span human mammary epithelial cell cultures and five immortalized but nontumorigenic human mammary epithelial cells. Vercoutter-Edouart et al. (21) also demonstrated that 14-3-3 sigma protein was strongly downregulated in the prototypic breast cancer cell lines MCF-7 and MDA-MB-231 and in primary breast carcinomas, compared with its expression in normal breast epithelial cells. Simooka et al. (22) investigated the timing of the loss of 14-3-3 sigma gene expression during breast tumorigenesis in vivo and detected 14-3-3 sigma expression in 92% of usual ductal hyperplasia lesions, and gradually decreased from 65% in ductal carcinoma in situ to 23% in invasive ductal carcinoma immunohistochemically. This appears to be a morphological reflection of the tumor suppressor function of 14-3-3 sigma, which was supported by Urano et al. (23).

Many studies have demonstrated that the loss of 14-3-3 sigma gene expression and the hypermethylation of its promoter are the most consistent molecular changes observed in breast cancer. The hypermethylation of CpG islands in the 14-3-3 sigma gene was detected in 91% (75/82) of breast tumors and was associated with a lack of gene expression (11). Luo et al. (24) reported that the frequency of methylation of the 14-3-3 sigma gene was 90% in 68 patients with sporadic breast cancer but only 18% (2/13) in hyperplastic samples, and no

Figure 1. Ganoderic acid D targets the protein 14-3-3 family in promoting cervical cancer. Ganoderic acid D, one of the major components of the Ganoderma triterpenes, inhibits the proliferation of HeLa cells and induces G2/M cell-cycle arrest and apoptosis by directly binding six isoforms of the 14-3-3 protein family, including 14-3-3 zeta/delta, 14-3-3 beta/alpha, 14-3-3 sigma, 14-3-3 theta, 14-3-3 gamma, and 14-3-3 epsilon.
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Hypermethylation was detected in normal breast tissue. The change in the methylation status of the 14-3-3 sigma gene correlated strongly with various types and grades of tumor and with lymph-node metastasis. The methylation of the 14-3-3 sigma gene also correlated inversely with its mRNA transcription and protein expression levels.

Umbricht et al. (25) presented evidence that hypermethylation-induced loss of 14-3-3 sigma expression is an early event in breast cancer and might also occur in apparently normal epithelium adjacent to a breast cancer. They detected the hypermethylation of the 14-3-3 sigma gene in 24 of 25 ductal carcinomas (96%), 15 of 18 (83%) breast ductal carcinomas in situ, and three of eight (38%) atypical breast hyperplasias. None of the five hyperplasias lacking atypia showed 14-3-3 sigma gene hypermethylation. Unexpectedly, the patients with breast cancer also showed sigma hypermethylation in adjacent histologically normal breast epithelium, whereas this was never observed in individuals with no evidence of breast cancer.

4.1.2. Changes in other 14-3-3 isoforms in breast carcinogenesis

In a study by Vercoutter-Edouart et al. (21), the levels of the alpha, beta, delta, and zeta isoforms of 14-3-3 were the same in both normal breast epithelial cells and breast cancer cell lines. However, many studies have reported higher expression of the 14-3-3 isoforms in breast cancer tissues. Using a proteomic approach, Liang et al. (26) found that the 14-3-3 zeta and 14-3-3 eta proteins were expressed at significantly higher levels in cancerous breast tissues than in normal breast tissues in patients with infiltrating ductal carcinoma, especially in a Malay cohort. Zang et al. (27) showed that 14-3-3 delta protein expression was significantly upregulated in the cells of metastatic ductal breast carcinoma compared with that in normal ductal epithelium. Song et al. (28) reported that the expression of the 14-3-3 gamma protein was significantly higher in breast cancer than in adjacent noncancerous mammary gland tissues when analyzed with immunohistochemistry and immunoblotting. The expression of 14-3-3 theta was also markedly higher in breast cancer tissues than in adjacent normal tissues in a hospital-based study of 216 breast cancer patients (29).

Kim et al. (30) demonstrated in vitro that MCF-7 breast cancer cells transfected with a construct expressing 14-3-3 beta displayed increased proliferation in response to estradiol, whereas a small interfering (si) construct, si-14-3-3 beta, reduced the estrogen-induced proliferation of the cells. They also showed that 14-3-3 beta functions as a positive regulator of estrogen receptor alpha (ERα), via a direct protein–protein interaction, in an estrogen-dependent manner, and then increases the expression of the endogenous target genes of ERα, leading to the proliferation of breast cancer cells. This suggests that 14-3-3 beta has oncogenic potential in breast cancer by binding to ERα and inducing its transcriptional activity (Figure 2).

4.2. Roles of 14-3-3 proteins in the clinicopathological features of breast cancer

Song et al. (28) reported that 14-3-3 gamma was strongly expressed in 79% (26/33) of large tumors but in only 44% (12/27) of small tumors. They also showed that 82% (18/22) of grade III tumors strongly expressed 14-3-3 gamma, whereas only 53% (20/38) of grade I and grade II tumors displayed strong 14-3-3 gamma expression. These findings indicate that high 14-3-3 gamma expression is significantly associated with tumor size and tumor grade. However, these researchers found no significant association between 14-3-3 gamma
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expression and other clinicopathological factors of the breast cancer patients, including age, histology, estrogen receptor, progesterone receptor, or axillary lymph-node metastasis.

Li et al. (31) reported that the expression of the 14-3-3 epsilon protein was upregulated in a highly metastatic variant of parental MDA-MB-435 breast cancer cells relative to its expression in the parental MDA-MB-435 cells, suggesting that the 14-3-3 epsilon protein plays an important role in breast cancer metastasis. They (29) also demonstrated that the overexpression of 14-3-3 theta correlated with an advanced tumor-node-metastasis (TNM) stage, lymph-node metastasis, and an ER-negative status. The knockdown of 14-3-3 theta expression in MDA-MB-231 breast cancer cells also inhibited metastasis in vitro. Similarly, an in vivo assay showed that 14-3-3 theta knockdown dramatically suppressed the growth of breast cancer xenografts and inhibited tumor cell metastasis in a model of lung metastasis.

In a study of infiltrating ductal breast cancer patients divided into four different cohorts according to their tumor stage and grade (stages II or III and grades II or III), Liang et al. (32) reported that 14-3-3 zeta protein was upregulated in all stages and grades compared with normal breast tissue: in 100% of stage II patients, 80% of stage III patients, 71% of grade II patients, and 100% of grade III patients. 14-3-3 protein eta was upregulated in 85% of stage II patients, 80% stage III patients, and 100% of grade III patients, although it was upregulated in only 57% of grade II patients. These findings indicate that both 14-3-3 zeta and 14-3-3 eta are significantly related to certain stages or grades of infiltrating ductal breast cancer and might be associated with the aggressiveness of the tumor before the tumor has spread.

4.3. Roles of 14-3-3 proteins in the diagnosis of breast cancer

In a study of 40 patients with cancerous and noncancerous breast tissues, Gheibi et al. (33) found that the methylation pattern of the 14-3-3 sigma promoter differed significantly in noncancerous and malignant breast tissues, but there was no marked correlation between methylation and age. When the methylation status of the 14-3-3 sigma promoter was examined in blood samples from the normal population, 20% (four of 20) were methylated and 80% (16 of 20) were unmethylated. These results indicate that 14-3-3 sigma has diagnostic utility for the early detection of breast cancer.

Rui et al. (34) showed that 14-3-3 sigma expression was downregulated in the sera of breast cancer patients compared with its expression in unaffected women. They used 14-3-3 sigma levels to classify an independent set of 104 masked serum samples, and found that the levels of 14-3-3 sigma on two-dimensional gels could completely distinguish the sera of breast cancer patients from those of unaffected subjects. Martínez-Galán et al. (35) analyzed the preoperative sera of 106 women with breast cancer, 34 with benign breast disease, and 74 with no evidence of breast disease (healthy controls). They demonstrated that the serum levels of the promoter-methylated 14-3-3 sigma gene differed significantly between breast cancer patients and healthy controls, and between the patients with benign breast disease and the healthy controls. They also showed that the hypermethylation of the 14-3-3 sigma gene distinguished breast cancer patients from healthy controls with a sensitivity of 75% and a specificity of 53%. However, several shortcomings of the study, including the frequent presence of the methylated 14-3-3 sigma gene in sera from women with benign breast disease, cast some doubt on the diagnostic utility of this measure for early cancer detection.

4.4. Roles of 14-3-3 proteins in the treatment of breast cancer

Ferguson et al. (11) demonstrated that breast cancer cells not expressing 14-3-3 sigma showed greater numbers of chromosomal breaks and gaps when exposed to 1 Gy gamma irradiation than the sigma-positive breast cancer cell line, MCF-7. They also found that 14-3-3-sigma-negative breast cancer cell lines accumulated more genetic damage during irradiation than the MCF-7 cell line, which is consistent with their failure of cell-cycle arrest in G2 in these cells in response to DNA damage. These results suggest that the loss of 14-3-3 sigma plays a role in determining the sensitivity of breast cancers to radiation therapy.

Bergamaschi et al. (37) investigated the role of 14-3-3 zeta in endocrine resistance in breast cancer and found that 14-3-3 zeta is a key predictive marker for the risk of endocrine therapy failure. The depletion of 14-3-3 zeta markedly increased apoptosis, reduced cell proliferation and receptor tyrosine kinase signaling, and importantly, reversed endocrine resistance. In their subsequent study, Bergamaschi and Katzenellenbogen (36) demonstrated that tamoxifen rapidly downregulates microRNA-451, which specifically targets 14-3-3 zeta, and with the consequent upregulation of the key survival factor 14-3-3 zeta. This is the mechanistic basis of the tamoxifen-associated development of endocrine resistance in ER-positive breast cancers. These findings suggest that therapeutic approaches that increase the expression of this tumor-suppressor-like microRNA should be considered to downregulate 14-3-3 zeta and enhance the effectiveness of endocrine therapies. Neal et al. (38) reported that MCF-7 cells treated with 14-3-3 zeta siRNA underwent increased cell death in response to 5-flourouracil or doxorubicin compared with the death of control-siRNA-treated cells, indicating that 14-3-3 zeta overexpression in breast cancers may contribute to their resistance to standard chemotherapies by conferring
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resistance to apoptosis. Therefore, targeting 14-3-3 zeta may sensitize cancer cells to chemotherapy.

Using two-dimensional gel electrophoresis and MALDI-TOF peptide mass fingerprinting, Chuthapisith et al. (39) compared the protein expression profiles of chemosensitive MCF-7 breast cancer cells and cells resistant to two different commonly used anticancer drugs ( Adriamycin and paclitaxel). 14-3-3 epsilon was overexpressed in both the adriamycin- and paclitaxel-resistant cells compared with its expression in drug-sensitive MCF-7 cells, and these findings were confirmed with immunoblotting. This provides further insight into the complex mechanisms of chemoresistance, and presents an attractive starting point for the identification of potential protein biomarkers to predict the response to chemotherapy in breast cancer in vivo.

Hodgkinson et al. (40) performed five comparative proteomic experiments using invasive with ER-positive ductal breast carcinomas ( luminal subtype). Using antibody microarrays, they showed that 88% (8/9) of the chemotherapy-resistant tumor samples stained positively for 14-3-3 theta/tau compared with 40% (9/22) of chemotherapy-sensitive tumor samples. These data suggest a potential role for 14-3-3 theta/tau as a predictive biomarker of neoadjuvant chemotherapy resistance in breast cancer, which is supported by another study by Hodgkinson et al. (41). Wang et al. (42) examined the tamoxifen response in MCF-7 breast cancer cells infected with 14-3-3-tau-expressing adenovirus or with empty vector. 14-3-3 tau dose-dependently alleviated the growth arrest exerted by tamoxifen. When 14-3-3 tau was depleted in MCF-7 cells and the tamoxifen response was measured, 14-3-3 tau depletion was shown to inhibited cell growth compared with the growth of the control cells. These findings indicate that 14-3-3 tau is potentially a novel therapeutic target that might be used to overcome hormonal resistance in breast cancer.

4.5. Roles of 14-3-3 proteins in the prognosis of breast cancer

Using a univariate analysis to determine the prognostic significance of age, histological type, lymph-node metastasis, and methylation status of the 14-3-3 sigma protein in 68 patients with sporadic breast cancer, Luo et al. (24) found that the methylation of the 14-3-3 sigma promoter did not affect the 5-year survival rate of these patients, and that only lymph-node metastasis was strongly associated with a poor outcome.

However, many studies have shown that the upregulated expression of 14-3-3 isoforms in breast cancer is significantly associated with a poor prognosis. Song et al. (28) reported that breast cancer patients with high 14-3-3 gamma expression had shorter overall survival rates than those with lower expression. A multivariate analysis also showed that 14-3-3 gamma expression is an independent predictor of overall survival. Li et al. (29) reported that breast cancer patients with high 14-3-3 theta expression had shorter overall survival and a higher rate of recurrence than those with low 14-3-3 theta expression. Neal et al. (38) showed that 14-3-3 zeta was overexpressed in 42% of primary breast carcinomas and that this overexpression, combined with ErbB2 overexpression and a positive lymph-node status, identified a subgroup of patients at high risk of distant metastasis. 14-3-3 zeta overexpression was also shown to be an independent prognostic factor for reduced disease-free survival in breast cancer patients. 14-3-3 tau is also frequently overexpressed in primary human breast cancers and correlates with shorter patient survival (42).

5. ROLES OF 14-3-3 PROTEINS IN OVARIAN TUMORS

5.1. Roles of 14-3-3 proteins in the biological behavior of ovarian cancer

Epithelial ovarian cancer is the leading cause of death from gynecological malignancies in the great majority of developed countries (43). Tumor-associated monocytes/macrophages are known to contribute to the immune–inflammatory cell environments of advanced epithelial ovarian carcinomas. Kobayashi et al. (44) demonstrated for the first time that 14-3-3 zeta is secreted by tumor-associated monocytes/macrophages into the ascites of epithelial ovarian carcinoma patients. Because 14-3-3 zeta is an adaptor protein produced and secreted by tumor-associated monocytes/macrophages at the tumor site, it seems likely that it plays a role in regulating the inflammatory pathways in the epithelial ovarian carcinoma microenvironment.

5.2. Roles of 14-3-3 proteins in the diagnosis of ovarian tumors

Kaneuchi et al. (45) demonstrated that the 14-3-3 sigma gene was methylated and inactivated in the ES-2 ovarian cell line derived from a clear-cell adenocarcinoma, and 14-3-3 sigma protein was not expressed in most ovarian clear-cell carcinoma tissues. However, they also demonstrated that the 14-3-3 sigma protein was expressed in significantly higher proportions of serous, endometrioid, and mucinous ovarian adenocarcinoma tissues. These findings suggest that the differential methylation status and expression of the 14-3-3 sigma gene characterizes the pathological differences among ovarian cancers. He et al. (46) reported that the average serum level of 14-3-3 zeta was significantly increased in patients with epithelial ovarian cancer compared with that in patients with benign gynecological diseases. The expression of 14-3-3 zeta was also associated with the degree of peritoneal metastasis of the cancer, the emergence of ascites, bilateral involvement, and the clinical stage and substage of the tumor. These data indicate that 14-3-3 zeta can be used as a biomarker to detect ovarian cancer during its occult metastatic stage.
and in the differential diagnosis of an unknown pelvic mass as early as possible.

Chen and Yang (47) used immunostaining to detect 14-3-3 sigma in 103 ovarian sex-cord stromal neoplasms. They found that ovarian granulosa cell tumors arising from the sex-cord stromal cells of the ovary and all steroid cell tumors were positive for 14-3-3 sigma. However, all Sertoli cell tumors, fibromas, thecomas, ovarian endometrial stromal sarcomas, and sex-cord stromal tumors (unclassified) were negative for 14-3-3 sigma. Therefore, it is possible that 14-3-3 sigma is a useful immunohistochemical marker for the differential diagnosis of ovarian granulosa cell tumors and steroid cell tumors.

5.3. Roles of 14-3-3 proteins in monitoring the treatment of ovarian cancers

Hatzipetros et al. (48) collected peripheral blood samples preoperatively from 13 patients with advanced stage (FIGO stages IIb–IIIc) epithelial ovarian cancer, who underwent radical surgery and six consecutive cycles of first-line chemotherapy (paclitaxel, carboplatin) at 21-day intervals. The levels of 14-3-3 zeta protein did not differ significantly between healthy postmenopausal subjects and patients with epithelial ovarian cancer following chemotherapy. This finding suggests that the levels of 14-3-3 zeta protein do not correlate reliably with the clinical behavior of epithelial ovarian cancer.

5.4. Roles of 14-3-3 proteins in the prognosis of ovarian cancer

Using tissue microarrays containing 192 samples of epithelial ovarian carcinoma, Mhawech-Fauceglia et al. (49) showed that the loss of 14-3-3 sigma expression was not associated with overall survival or disease-free survival, suggesting that 14-3-3 sigma does not have prognostic value as a biomarker for predicting patient outcomes. In contrast, Ravi et al. (50) found that metastatic ovarian tumors frequently overexpressed 14-3-3 sigma protein, which in conjunction with phosphorylated retinoblastoma, resulted in a poor prognosis. Furthermore, the expression of 14-3-3 sigma was statistically significantly upregulated in metastatic primary ovarian tumors compared with normal tissues or malignant ovarian tumors with no metastasis. This suggests that 14-3-3 sigma expression has prognostic implications for patients with ovarian tumors.

6. ROLES OF 14-3-3 PROTEINS IN ENDOMETRIAL CANCER

Nakayama et al. (51) showed that the hypomethylation of the 14-3-3 sigma gene causes it to be overexpressed in the normal endometrium in the mid- to late-secretory phase. In contrast, they also reported that the hypermethylation of the 14-3-3 sigma gene reduces its expression in low-grade endometrioid adenocarcinomas, and that this suppression increases significantly with increasing histological grade as hypomethylation increases. The expression of 14-3-3 sigma was low or moderate in nonneoplastic lesions, including various hyperplastic endometria and atrophic endometria. These findings suggest that endometrial adenocarcinoma arises from a background endometrium with low 14-3-3 sigma expression, and that 14-3-3 sigma expression increases throughout tumorigenesis and cancer progression.

Lee et al. (52) reported a transforming 14-3-3 oncoprotein resulting from a highly recurrent genetic mechanism in high-grade endometrial stromal sarcoma, a clinically aggressive form of uterine sarcoma. They attributed the 14-3-3 oncoprotein to a t(10;17) genomic rearrangement, leading to the fusion of 14-3-3 epsilon (YWHAE) and either of two nearly identical FAM22 family members (FAM22A or FAM22B). They confirmed the expression of the YWHAE–FAM22 fusion oncoprotein in t(10;17)-bearing frozen tumor and cell line samples. The absolute specificity of the YWHAE–FAM22A/B genetic rearrangement for high-grade endometrial stromal sarcoma was demonstrated, and no fusions were detected in other uterine or nonuterine mesenchymal tumors. The discovery of such a unique oncogenic mechanism has biological, diagnostic, and therapeutic implications.

7. ROLES OF 14-3-3 PROTEINS IN VULVAR CANCER

In our previous study (53), we showed that 14-3-3 gamma was markedly downregulated in leiomyoma tissues compared with its expression in the normal myometrium, suggesting a possible role for 14-3-3 gamma in the cause and growth of leiomyomas. Wang et al. (54) demonstrated that the expression of 14-3-3 sigma was significantly reduced in leiomyomas compared with that in the normal myometrium and correlated negatively with the estrogen and progesterone receptors. The downregulation of 14-3-3 sigma in leiomyoma suggests that 14-3-3 sigma plays a role in tumorigenesis, and may be involved in the upregulation of the estrogen and progesterone receptors.

8. ROLES OF 14-3-3 PROTEINS IN VULVAR CANCER

Wang et al. (55) investigated the expression of 14-3-3 sigma in 302 patients with vulvar squamous cell carcinoma and its association with their clinicopathological characteristics and clinical outcomes. 14-3-3 sigma protein was highly expressed in the cytoplasm but not in the cell nuclei in all 11 normal tissues tested, whereas 72% and 59% of the vulvar carcinomas showed high expression of 14-3-3 sigma in the cytoplasm and nucleus, respectively, indicating that the subcellular distribution of the 14-3-3 sigma
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protein differs in the cells of normal and cancerous tissues. Strong expression of 14-3-3 sigma protein in the cytoplasm, nucleus, or both in the vulvar carcinomas was significantly associated with a large tumor diameter and deep invasion. However, 14-3-3 sigma expression in neither the cytoplasm nor the nucleus was associated with disease-specific survival.

In another study, Wang et al. (56) showed that high cytoplasmic levels of the 14-3-3 beta, gamma, epsilon, zeta, and eta isoforms were associated with advanced disease and aggressive tumor characteristics, including a high FIGO stage, the presence of lymph-node metastasis, a large tumor diameter, deeper invasion, and/or a low histological grade. They also demonstrated a significant correlation between the low expression of 14-3-3 tau and larger tumor diameters. However, the low cytoplasmic expression of 14-3-3 tau did not correlate with disease-specific survival. These results suggest that the 14-3-3 beta, gamma, epsilon, zeta, eta, and tau isoforms are involved in the progression of vulvar carcinoma. High cytoplasmic levels of 14-3-3 beta and epsilon were also shown to be related to poor disease-specific survival.

9. CONCLUSIONS

The 14-3-3 proteins are found in many tissues and their presence in gynecological tumors has been established for many years. However, studies of the 14-3-3 proteins in human cancers have produced contradictory results, attributable to the diverse roles of the seven isoforms in cancer. The roles of the 14-3-3 proteins in gynecological tumors remain unclear because both tumor-suppressing and tumor-promoting activities have been attributed to them. In this context, it appears that only 14-3-3 sigma has tumor-suppressing activity, and is not associated with a poor prognosis in cervical, ovarian, or vulvar cancer. However, 14-3-3 sigma correlates with a poor prognosis in endometrial cancer. The other isoforms, including beta, gamma, epsilon, zeta, and eta, have also been reported to correlate significantly with poor prognoses in breast and vulvar cancer. Mounting evidence indicates that the 14-3-3 proteins promote or inhibit tumor growth through diverse mechanisms, including the hypermethylation of the 14-3-3 genes. Understanding the roles and modes of action of the 14-3-3 proteins in specific gynecological tumors may allow us to formulate more-effective targeted therapies in the future. Indeed, the 14-3-3 proteins might offer new targets for the adjuvant treatment of tumors. Further studies will require the establishment of whole-animal models and the generation of transgenic and knockout mouse models for each 14-3-3 isoform.

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**Abbreviations:** HPV, human papillomavirus; CIN, cervical intraepithelial neoplasia; FIGO, Federation of Gynecology and Obstetrics

**Key Words:** HPV, 14-3-3 protein, Gynecological Tumors, Review

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