[Frontiers in Bioscience, Landmark, 20, 934-945, June 1, 2015]

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

Wenwen Zhang[1], Qi Shen[1], Miaomiao Chen[1], Ying Wang[1], Qingfeng Zhou[1], Xuejiao Tao[1], Xueqiong Zhu[1]

[1]Department of Obstetrics and Gynecology, the Second Affiliated Hospital of Wenzhou Medical University, Wenzhou 325027, China

TABLE OF CONTENTS

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 gyncyological 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 G_2/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 G_2/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

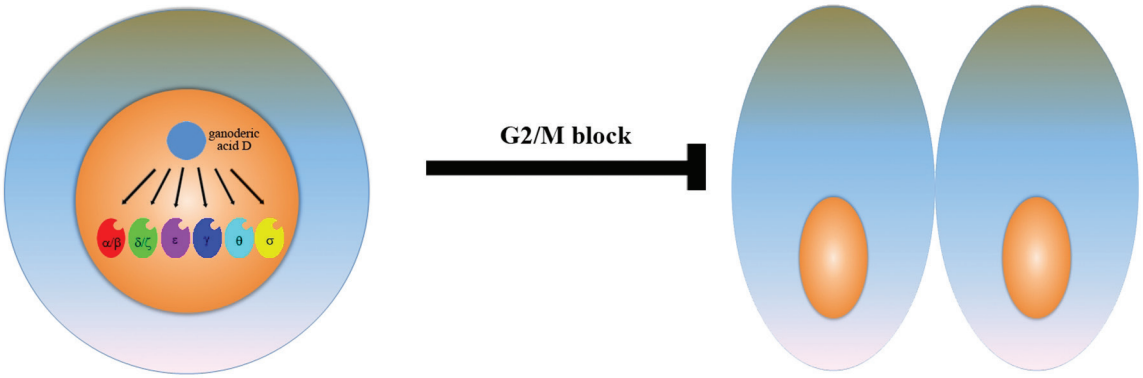

Figure 1. Ganoderic acid D targets the protein 14-3-3 family in promoting cervical cancer. Ganoderic acid D, one of the major components of the *Ganoderma* triterpenes, inhibits the proliferation of HeLa cells and induces G_2/M cell-cycle arrest and apoptosis by directly binding six isoforms of the 14-3-3 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.

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* triterpenes.

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

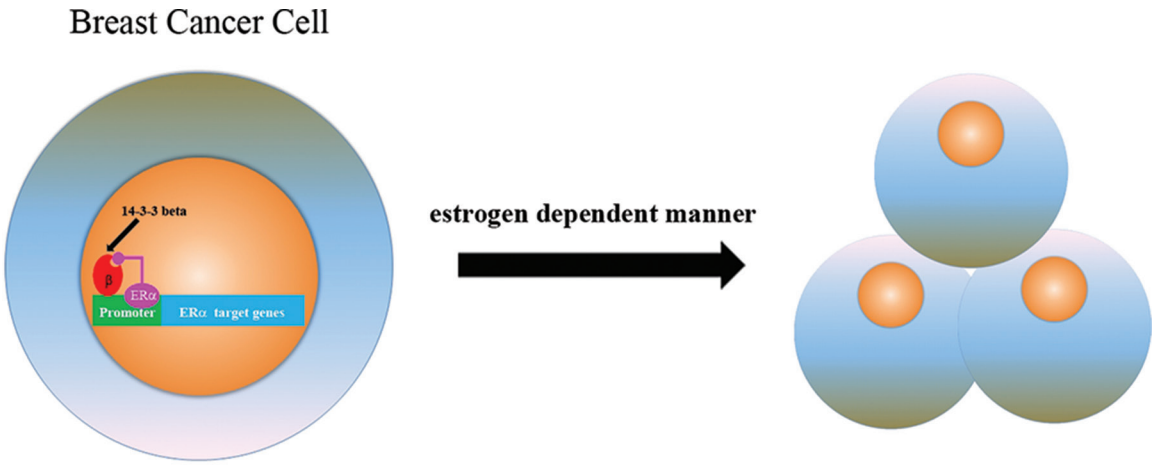

Figure 2. Protein 14-3-3 beta promotes breast cancer by upregulating the expression of estrogen receptor alpha (ERa) and its target genes. Protein 14-3-3 beta positively regulates the expression of ERa, in an estrogen-dependent manner, through a direct protein–protein interaction, and then increases the expressions of the endogenous target genes of ERa, leading to the proliferation of breast cancer cells.

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 (ERa), *via* a direct protein–protein interaction, in an estrogen-dependent manner, and then increases the expression of the endogenous target genes of ERa, leading to the proliferation of breast cancer cells. This suggests that 14-3-3 beta has oncogenic potential in breast cancer by binding to ERa 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

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 tissues: 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 G_2 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

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 UTERINE LEIOMYOMA

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

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 substage, 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 nuclear expression of 14-3-3 tau and larger tumor diameters. However, the low nuclear 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 mechanism, 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.

10. ACKNOWLEDGEMENTS

This work was sponsored by a grant from the National Natural Science Foundation of China (No. 81171926) and sponsored by Zhejiang Provincial Program for the Cultivation of High-level Innovative Health talents.

11. REFERENCES

1. Aitken A, Collinge DB, van Heusden BP, Isobe T, Roseboom PH, Rosenfeld G, Soll J: 14-3-3 proteins: a highly conserved, widespread family of eukaryotic proteins. *Trends Biochem Sci* 17, 498-501 (1992)
DOI: 10.1016/0968-0004(92)90339-B

2. Wang W, Shakes DC: Molecular evolution of the 14-3-3 protein family. *J Mol Evol* 43, 384-398 (1996)
DOI: 10.1007/BF02339012

3. Hermeking H: The 14-3-3 cancer connection. *Nat Rev Cancer* 3, 931-943 (2003)
DOI: 10.1038/nrc1230

4. Meek SE, Lane WS, Piwnica-Worms H: Comprehensive proteomic analysis of interphase and mitotic 14-3-3-binding proteins. *J Biol Chem* 279, 32046-32054 (2004)
DOI: 10.1074/jbc.M403044200

5. van Hemert MJ, Steensma HY, van Heusden GP: 14-3-3 proteins: key regulators of cell division, signalling and apoptosis. *Bioessays* 23, 936-946 (2001)
DOI: 10.1002/bies.1134

6. Martinez JD: 14-3-3 proteins: do they have a role in human cancer? *Future Oncol* 1, 631-633 (2005)
DOI: 10.2217/14796694.1.5.631

7. Morrison DK: The 14-3-3 proteins: integrators of diverse signaling cues that impact cell fate and cancer development. *Trends Cell Biol* 19, 16-23 (2009)
DOI: 10.1016/j.tcb.2008.10.003

8. Tzivion G, Gupta VS, Kaplun L, Balan V: 14-3-3 proteins as potential oncogenes. *Semin Cancer Biol* 16, 203-213 (2006)
DOI: 10.1016/j.semcancer.2006.03.004

9. Li Z, Liu JY, Zhang JT: 14-3-3sigma, the double-edged sword of human cancers. *Am J Transl Res* 1, 326–340 (2009)
Doi not found.

10. Holm R, Ali T, Svendsrud DH, Nesland JM, Kristensen GB, Lyng H: Expression of 14-3-3sigma in cervical squamous cell carcinomas:

relationship with clinical outcome. *Oncol Rep* 22, 11-15 (2009)
DOI: 10.3892/or_00000399

11. Ferguson AT, Evron E, Umbricht CB, Pandita TK, Chan TA, Hermeking H, Marks JR, Lambers AR, Futreal PA, Stampfer MR, Sukumar S: High frequency of hypermethylation at the 14-3-3 sigma locus leads to gene silencing in breast cancer. *Proc Natl Acad Sci U S A* 97, 6049-6054 (2000)
DOI: 10.1073/pnas.100566997

12. Laronga C, Yang HY, Neal C, Lee MH: Association of the cyclin-dependent kinases and 14-3-3 sigma negatively regulates cell cycle progression. *J Biol Chem* 275, 23106-23112 (2000)
DOI: 10.1074/jbc.M905616199

13. Higareda-Almaraz JC, Enríquez-Gasca Mdel R, Hernández-Ortiz M, Resendis-Antonio O, Encarnación-Guevara S: Proteomic patterns of cervical cancer cell lines, a network perspective. *BMC Syst Biol* 5, 96 (2011)
DOI: 10.1186/1752-0509-5-96

14. Boon SS, Banks L: High-risk human papillomavirus E6 oncoproteins interact with 14-3-3ζ in a PDZ binding motif-dependent manner. *J Virol* 87, 1586-1595 (2013)
DOI: 10.1128/JVI.02074-12

15. Syrjänen S, Naud P, Sarian L, Derchain S, Roteli-Martins C, Longatto-Filho A, Tatti S, Branca M, Erzen M, Hammes LS, Costa S, Syrjänen K: Up-regulation of 14-3-3sigma (Stratifin) is associated with high-grade CIN and high-risk human papillomavirus (HPV) at baseline but does not predict outcomes of HR-HPV infections or incident CIN in the LAMS study. *Am J Clin Pathol* 133, 232-240 (2010)
DOI: 10.1309/AJCP49DOITYDCTQJ

16. Kaefer CM, Milner JA: The role of herbs and spices in cancer prevention. *J Nutr Biochem* 19, 347-361 (2008)
DOI: 10.1016/j.jnutbio.2007.11.003

17. Liu Q, Peng YB, Qi LW, Cheng XL, Xu XJ, Liu LL, Liu EH, Li P: The Cytotoxicity Mechanism of 6-Shogaol-Treated HeLa Human Cervical Cancer Cells Revealed by Label-Free Shotgun Proteomics and Bioinformatics Analysis. *Evid Based Complement Alternat Med* 2012, 278652 (2012)
Doi not found.

18. Yue QX, Cao ZW, Guan SH, Liu XH, Tao L, Wu WY, Li YX, Yang PY, Liu X, Guo DA: Proteomics characterization of the cytotoxicity mechanism of ganoderic acid D and computer-automated estimation of the possible drug target network. *Mol Cell Proteomics* 7, 949-961 (2008)
DOI: 10.1074/mcp.M700259-MCP200

19. Yue QX, Song XY, Ma C, Feng LX, Guan SH, Wu WY, Yang M, Jiang BH, Liu X, Cui YJ, Guo DA: Effects of triterpenes from Ganoderma lucidum on protein expression profile of HeLa cells. *Phytomedicine* 17, 606-613 (2010)
DOI: 10.1016/j.phymed.2009.12.013

20. Moreira JM, Ohlsson G, Rank FE, Celis JE: Down-regulation of the tumor suppressor protein 14-3-3sigma is a sporadic event in cancer of the breast. *Mol Cell Proteomics* 4, 555-569 (2005)
DOI: 10.1074/mcp.M400205-MCP200

21. Vercoutter-Edouart AS, Lemoine J, Le Bourhis X, Louis H, Boilly B, Nurcombe V, Révillion F, Peyrat JP, Hondermarck H: Proteomic analysis reveals that 14-3-3sigma is down-regulated in human breast cancer cells. *Cancer Res* 61, 76-80 (2001)
Doi not found.

22. Simooka H, Oyama T, Sano T, Horiguchi J, Nakajima T: Immunohistochemical analysis of 14-3-3 sigma and related proteins in hyperplastic and neoplastic breast lesions, with particular reference to early carcinogenesis. *Pathol Int* 54, 595-602 (2004)
DOI: 10.1111/j.1440-1827.2004.01668.x

23. Urano T, Saito T, Tsukui T, Fujita M, Hosoi T, Muramatsu M, Ouchi Y, Inoue S: Efp targets 14-3-3 sigma for proteolysis and promotes breast tumour growth. *Nature* 417, 871-875 (2002)
DOI: 10.1038/nature00826

24. Luo J, Feng J, Lu J, Wang Y, Tang X, Xie F, Li W: Aberrant methylation profile of 14-3-3 sigma and its reduced transcription/expression levels in Chinese sporadic female breast carcinogenesis. *Med Oncol* 27, 791-797 (2010)
DOI: 10.1007/s12032-009-9287-8

25. Umbricht CB, Evron E, Gabrielson E, Ferguson A, Marks J, Sukumar S: Hypermethylation of 14-3-3 sigma (stratifin) is an early event in breast cancer. *Oncogene* 20, 3348-3353 (2001)

DOI: 10.1038/sj.onc.1204438

26. Liang S, Singh M, Gam LH: The differential expression of aqueous soluble proteins in breast normal and cancerous tissues in relation to ethnicity of the patients; Chinese, Malay and Indian. *Dis Markers* 28, 149-165 (2010)
DOI: 10.1155/2010/704630

27. Zang L, Palmer Toy D, Hancock WS, Sgroi DC, Karger BL: Proteomic analysis of ductal carcinoma of the breast using laser capture microdissection, LC-MS, and 16O/18O isotopic labeling. *J Proteome Res* 3, 604-612 (2004)
DOI: 10.1021/pr034131l

28. Song Y, Yang Z, Ke Z, Yao Y, Hu X, Sun Y, Li H, Yin J, Zeng C: Expression of 14-3-3γ in patients with breast cancer: correlation with clinicopathological features and prognosis. *Cancer Epidemiol* 36, 533-536 (2012)
DOI: 10.1016/j.canep.2012.05.003

29. Li N, Wang H, Fan J, Tong C, Yang J, Wei H, Yi J, Ling R: Overexpression of 14-3-3θ promotes tumor metastasis and indicates poor prognosis in breast carcinoma. *Oncotarget* 5, 249-257. (2014)
Doi not found.

30. Kim Y, Kim H, Jang SW, Ko J: The role of 14-3-3β in transcriptional activation of estrogen receptor α and its involvement in proliferation of breast cancer cells. *Biochem Biophys Res Commun* 414, 199-204 (2011)
DOI: 10.1016/j.bbrc.2011.09.056

31. Li DQ, Wang L, Fei F, Hou YF, Luo JM; Wei-Chen, Zeng R, Wu J, Lu JS, Di GH, Ou ZL, Xia QC, Shen ZZ, Shao ZM: Identification of breast cancer metastasis-associated proteins in an isogenic tumor metastasis model using two-dimensional gel electrophoresis and liquid chromatography-ion trap-mass spectrometry. *Proteomics* 6, 3352-3368 (2006)
DOI: 10.1002/pmic.200500617

32. Liang S, Singh M, Gam LH: The differential expression of aqueous soluble proteins in breast normal and cancerous tissues in relation to stage and grade of patients. *J Biomed Biotechnol* 2010, 516469 (2010)
DOI: 10.1155/2010/516469

33. Gheibi A, Kazemi M, Baradaran A, Akbari M, Salehi M: Study of promoter methylation pattern of 14-3-3 sigma gene in normal and cancerous tissue of breast: A potential biomarker for detection of breast cancer in patients. *Adv Biomed Res* 1, 80 (2012)
Doi not found.

34. Rui Z, Jian-Guo J, Yuan-Peng T, Hai P, Bing-Gen R: Use of serological proteomic methods to find biomarkers associated with breast cancer. *Proteomics* 3, 433-439 (2003)
DOI: 10.1002/pmic.200390058

35. Martínez-Galán J, Torres B, Del Moral R, Muñoz-Gámez JA, Martín-Oliva D, Villalobos M, Núñez MI, Luna Jde D, Oliver FJ, Ruiz de Almodóvar JM: Quantitative detection of methylated ESR1 and 14-3-3-sigma gene promoters in serum as candidate biomarkers for diagnosis of breast cancer and evaluation of treatment efficacy. *Cancer Biol Ther* 7, 958-965 (2008)
DOI: 10.4161/cbt.7.6.5966

36. Bergamaschi A, Katzenellenbogen BS: Tamoxifen downregulation of miR-451 increases 14-3-3ζ and promotes breast cancer cell survival and endocrine resistance. *Oncogene* 31, 39-47 (2012)
DOI: 10.1038/onc.2011.223

37. Bergamaschi A, Christensen BL, Katzenellenbogen BS: Reversal of endocrine resistance in breast cancer: interrelationships among 14-3-3ζ, FOXM1, and a gene signature associated with mitosis. *Breast Cancer Res* 13, R70 (2011)
DOI: 10.1186/bcr2913

38. Neal CL, Yao J, Yang W, Zhou X, Nguyen NT, Lu J, Danes CG, Guo H, Lan KH, Ensor J, Hittelman W, Hung MC, Yu D: 14-3-3zeta overexpression defines high risk for breast cancer recurrence and promotes cancer cell survival. *Cancer Res* 69, 3425-3432 (2009)
DOI: 10.1158/0008-5472.CAN-08-2765

39. Chuthapisith S, Layfield R, Kerr ID, Hughes C, Eremin O: Proteomic profiling of MCF-7 breast cancer cells with chemoresistance to different types of anti-cancer drugs. *Int J Oncol* 30, 1545-1551 (2007)
Doi not found.

40. Hodgkinson VC, ELFadl D, Agarwal V, Garimella V, Russell C, Long ED, Fox JN, McManus PL, Mahapatra TK, Kneeshaw PJ, Drew PJ, Lind MJ, Cawkwell L: Proteomic

identification of predictive biomarkers of resistance to neoadjuvant chemotherapy in luminal breast cancer: a possible role for 14-3-3 theta/tau and tBID? *J Proteomics* 75, 1276-1283 (2012)
DOI: 10.1016/j.jprot.2011.11.005

41. Hodgkinson VC, Agarwal V, ELFadl D, Fox JN, McManus PL, Mahapatra TK, Kneeshaw PJ, Drew PJ, Lind MJ, Cawkwell L: Pilot and feasibility study: comparative proteomic analysis by 2-DE MALDI TOF/TOF MS reveals 14-3-3 proteins as putative biomarkers of response to neoadjuvant chemotherapy in ER-positive breast cancer. *J Proteomics* 75, 2745-2752 (2012)
DOI: 10.1016/j.jprot.2012.03.049

42. Wang B, Liu K, Lin HY, Bellam N, Ling S, Lin WC: 14-3-3Tau regulates ubiquitin-independent proteasomal degradation of p21, a novel mechanism of p21 downregulation in breast cancer. *Mol Cell Biol* 30, 1508-1527 (2010)
DOI: 10.1128/MCB.01335-09

43. Cannistra SA, Bast RC Jr, Berek JS, Bookman MA, Crum CP, DePriest PD, Garber JE, Koh WJ, Markman M, McGuire WP 3rd, Rose PG, Rowinsky EK, Rustin GJ, Skates SJ, Vasey PA, King L: Progress in the management of gynecologic cancer: consensus summary statement. *J Clin Oncol* 21, 129s-132s (2003)
DOI: 10.1200/JCO.2003.04.003

44. Kobayashi R, Deavers M, Patenia R, Rice-Stitt T, Halbe J, Gallardo S, Freedman RS: 14-3-3 zeta protein secreted by tumor associated monocytes/macrophages from ascites of epithelial ovarian cancer patients. *Cancer Immunol Immunother* 58, 247-258 (2009)
DOI: 10.1007/s00262-008-0549-7

45. Kaneuchi M, Sasaki M, Tanaka Y, Shiina H, Verma M, Ebina Y, Nomura E, Yamamoto R, Sakuragi N, Dahiya R: Expression and methylation status of 14-3-3 sigma gene can characterize the different histological features of ovarian cancer. *Biochem Biophys Res Commun* 316, 1156-1162 (2004)
DOI: 10.1016/j.bbrc.2004.02.171

46. He Y, Wu X, Liu X, Yan G, Xu C: LC-MS/MS analysis of ovarian cancer metastasis-related proteins using a nude mouse model: 14-3-3 zeta as a candidate biomarker. *J Proteome Res* 9, 6180-6190 (2010)
DOI: 10.1021/pr100822v

47. Chen L, Yang B: 14-3-3 sigma is a useful immunohistochemical marker for diagnosing ovarian granulosa cell tumors and steroid cell tumors. *Int J Gynecol Pathol* 32, 156-162 (2013)
DOI: 10.1097/PGP.0b013e31825a0353

48. Hatzipetros I, Gocze P, Koszegi T, Jaray A, Szereday L, Polgar B, Farkas N, Farkas B: Investigating the clinical potential for 14-3-3 zeta protein to serve as a biomarker for epithelial ovarian cancer. *J Ovarian Res* 6, 79 (2013)
DOI: 10.1186/1757-2215-6-79

49. Mhawech-Fauceglia P, Herrmann FR, Andrews C, South S, Beck A, Lele S, Odunsi K: 14-3-3sigma expression and prognostic value in patients with epithelial ovarian carcinoma: a high throughput tissue microarray analysis. *Eur J Surg Oncol* 35, 763-767 (2009)
DOI: 10.1016/j.ejso.2008.10.014

50. Ravi D, Chen Y, Karia B, Brown A, Gu TT, Li J, Carey MS, Hennessy BT, Bishop AJ: 14-3-3 σ expression effects G2/M response to oxygen and correlates with ovarian cancer metastasis. *PLoS One* 6, e15864 (2011)
DOI: 10.1371/journal.pone.0015864

51. Nakayama H, Sano T, Motegi A, Oyama T, Nakajima T: Increasing 14-3-3 sigma expression with declining estrogen receptor alpha and estrogen-responsive finger protein expression defines malignant progression of endometrial carcinoma. *Pathol Int* 55, 707-715 (2005)
DOI: 10.1111/j.1440-1827.2005.01900.x

52. Lee CH, Ou WB, Mariño-Enriquez A, Zhu M, Mayeda M, Wang Y, Guo X, Brunner AL, Amant F, French CA, West RB, McAlpine JN, Gilks CB, Yaffe MB, Prentice LM, McPherson A, Jones SJ, Marra MA, Shah SP, van de Rijn M, Huntsman DG, Dal Cin P, Debiec-Rychter M, Nucci MR, Fletcher JA: 14-3-3 fusion oncogenes in high-grade endometrial stromal sarcoma. *Proc Natl Acad Sci U S A* 109, 929-934 (2012)
DOI: 10.1073/pnas.1115528109

53. Lv J, Zhu X, Dong K, Lin Y, Hu Y, Zhu C: Reduced expression of 14-3-3 gamma in uterine leiomyoma as identified by proteomics. *Fertil Steril* 90, 1892-1898 (2008)
DOI: 10.1016/j.fertnstert.2007.08.039

54. Wang L, Huang H, Liu D, Fang S, Xian Y, Zhou J, Zuo Y, Wang F, Huang O, He M: Evaluation of 14-3-3 protein family levels and associated receptor expression of estrogen and progesterone in human uterine leiomyomas. *Gynecol Endocrinol* 28, 665-668 (2012) DOI: 10.3109/09513590.2012.650768

55. Wang Z, Tropè CG, Suo Z, Trøen G, Yang G, Nesland JM, Holm R: The clinicopathological and prognostic impact of 14-3-3 sigma expression on vulvar squamous cell carcinomas. *BMC Cancer* 8, 308 (2008) DOI: 10.1186/1471-2407-8-308

56. Wang Z, Nesland JM, Suo Z, Trope CG, Holm R: The prognostic value of 14-3-3 isoforms in vulvar squamous cell carcinoma cases: 14-3-3β and ε are independent prognostic factors for these tumors. *PLoS One* 6, e24843 (2011) DOI: 10.1371/journal.pone.0024843

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

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

Send correspondence to: Xueqiong Zhu, No. 109 Xueyuan Xi Road, Department of Obstetrics and Gynecology, the Second Affiliated Hospital of Wenzhou Medical University, Wenzhou, Zhejiang, 325027, China, Tel: 86-577-88002796, Fax: 86-577-88832693, E-mail: zjwzzxq@yeah.net