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Nestin: A biomarker of aggressive uterine cancers☆,☆☆,☆☆☆

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HIGHLIGHTS

- High uterine cancer expression of nestin indicated worse PFS, CSS and OS.
- Nestin predicted worse PFS following no therapy or radiation but not chemotherapy.
- Nestin also predicted shorter PFS in lower risk, early stage, type I and ER+ disease.

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ABSTRACT

Objective. Evidence of potential prognostic and predictive value for nestin was investigated in well-annotated uterine cancers (UCs).

Methods. Nestin expression and previously-published biomarkers were evaluated by immunohistochemistry (IHC) in UC tissue microarrays. Biomarkers were categorized as low vs. high, and nestin was cut at 10% positive staining. Relationship between nestin and clinicopathologic factors, biomarkers and outcome were evaluated using exact/log-rank testing or logistic/Cox modeling.

Results. There were 323 eligible cases, 34% had advanced stage disease, 37% had type II disease, and 5% were carcinosarcomas. High nestin, observed in 19% of cases, was more common in advanced vs. early stage disease, type II cancers or uterine carcinosarcoma vs. type I cancers, grade 3 disease, positive lymphovascular space invasion (LVSI) and tumors >6 cm ($p < 0.05$). Nestin was inversely correlated with ER, PR and TFF3, and correlated with p53 and IMP3. Women with high vs. low nestin had worse progression-free survival (PFS) and cancer-specific survival overall, and worse PFS in the subset who received no adjuvant therapy or radiation, or had early stage, type I disease or tumors with both low and high ER, PR, TFF3, PTEN, p53 or IMP3. The relationship between nestin and PFS was independent of stage, LVSI and risk categorization but not type of UC.

Conclusions. High nestin was more common in UCs with aggressive features and poor outcome. Nestin may represent a predictive biomarker for treatment selection for patients previously considered to be lower risk and a candidate for no or radiation-based adjuvant therapy, and compliment ER/PR testing.

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1. Introduction

Uterine cancer is the fifth leading cause of cancer in women worldwide and the most common gynecologic cancer in the United States (U.S.), with diagnosis in over 350,000 and 54,000, and deaths in 68,000 and 10,000 women each year in the world and U.S., respectively [1,2]. Since the 1970s, uterine cancer incidence has steadily increased while five-year overall survival has decreased [3]. The obesity epidemic and increasing average lifespans are likely associated with these trends.

Uterine cancers are currently classified by histology. The majority of uterine cancers are considered type I disease, have a good prognosis, and are most closely associated with unopposed endogenous or exogenous estrogen [4]. Type II cancers include grade 3 endometrioid, serous, and clear cell [5], and the remaining cancers of the uterine corpus encompass carcinosarcomas and other sarcomas. These non-low-grade endometrioid cancers are typically more virulent and account for a disproportionate number of uterine cancer deaths [6,7]. The Cancer Genome Atlas (TCGA) Research Network and other groups are defining molecular distinctions and similarities between the different uterine cancer subtypes [8] and these biomarkers have been shown to have predictive [9] and biologic value [9–14].

Clinical management is based on the above classifications, which take into account histology and grade, in conjunction with known risk factors such as surgical stage, lymphovascular space invasion (LVSI), tumor size, and age at diagnosis [12,15], but no effective therapies exist for aggressive disease. Current risk stratification falls short of preventing over-treatment in many patients who would have never recurred and under-treatment in many who may ultimately die of recurrent or progressive disease. Given the prevalence of low risk uterine cancer patients, identification of biomarkers that aid in risk stratification are needed.

Nestin, a class VI intermediate filament protein first described as a neural stem marker [16], regulates the TGFβ pathway [17,18] and plays an important role in cancer cell migration, invasion, and metastasis by interacting with vimentin or desmin and modulating the expression of actin and cell adhesion molecules [17,19–21]. *In vitro* studies have shown that knockdown of nestin inhibits migration, invasion, and metastasis of cancer cells [17,22]. Nestin also plays a key role in angiogenesis [19]. Though unexplored in uterine cancer, nestin expression has been reported in ganglioglioma, ovarian, head and neck, prostate, bladder, and pancreatic cancers, and is a prognostic indicator of poor survival in many cancers [20,21]. In the current investigation, we examined the potential prognostic and predictive value of nestin expression levels in uterine cancer by studying associations with established prognostic clinical factors and biomarkers as well as measures of clinical outcome, including progression-free (PFS) and cancer-specific survival (CSS).

2. Materials and methods

2.1. Uterine cancer patients with formalin-fixed and paraffin-embedded tumor

The University of Southern California archives and database were searched for uterine cancer patients treated from 1998 to 2010. All tissue specimens were collected and medical records reviewed under approved protocols from the Institutional Review Board (IRB). Endometrioid, clear cell, serous, or carcinosarcoma patients with de-identified clinical, outcome, and evaluable nestin data were eligible. Treatment decisions were made by physician and patients. For the purposes of this analysis, stage I & II disease were categorized as early stage uterine cancer, and stage III & IV disease were categorized as advanced stage uterine cancer. In addition, grade 1 and 2 (G1/G2) endometrioid carcinomas were categorized as type I uterine cancer. Serous adenocarcinomas, clear cell carcinomas, and grade 3 (G3) endometrioid carcinomas were categorized as type II uterine cancer. Carcinosarcomas including homologous, heterologous or not specified were evaluated collectively as a separate subtype.

Risk categorization represented a modification of the Keys GOG-99 and PORTEC classification [15,23] with myometrial invasion categorized at 50%. The low-risk group included patients with G1 or G2 endometrioid endometrial adenocarcinoma with stage IA disease (<50% myometrial invasion) and negative LVSI. The intermediate-risk patients with stage I or II disease were further subdivided into low- vs. high-intermediate risk groups based on age at diagnosis (<50, 50 to 69, 70+ years old) and other clinical risk factors including positive LVSI, deep myometrial invasion (>50%), and G2/G3 disease. High-intermediate risk patients diagnosed at 70+ years of age needed one other clinical risk factor, at 50 to 69 years of age needed two other clinical risk factors and at <50 years of age needed all three other clinical risk factors. Patients not meeting the high-intermediate risk criteria within the intermediate risk group were classified as low-intermediate risk. The high-risk group included patients with stage III/IV disease as well as those with stage I/II disease with a non-endometrioid histologic subtypes such as serous adenocarcinomas (with or without the papillary designation), clear cell carcinomas, and carcinosarcoma. For analysis purposes, patients with low and low-intermediate risk groups were aggregated into a lower risk category, and patients with high-intermediate and high risk were combined into a higher risk category.

2.2. Tissue microarrays

Uterine cancer tissue microarrays (TMAs) were constructed using archival tissue from eligible patients as previously described [24]. Briefly, after carefully selecting the morphologically representative region from the hematoxylin & eosin (H&E)-stained section, 0.6 mm cores were punched from the individual donor formalin-fixed, paraffin-embedded blocks, and incorporated into the TMA paraffin receiver blocks. To account for tumor heterogeneity, cores were sampled from three different areas of each tumor. One section from each TMA block was stained with H&E to confirm the presence of the tumor by light microscopy.

2.3. Nestin immunohistochemistry (IHC) assay and scoring criteria

Four μm thick sections prepared on positively charged slides from tumor blocks from individual patients or TMA blocks were deparaffinized and pretreated in citrate buffer pH 6.0 for 20 min. Sections were cooled 20 min and incubated 10 min at ambient temperature in 3% H_2O_2 to quench endogenous peroxidase activity. Blocking was performed using serum-free protein block (Dakocytomation, Carpenteria, CA) for 30 min. Slides were loaded on a Dakocytomation autostainer and an anti-nestin antibody (1:50, LifeScience, Memphis, TN; monoclonal) was applied for 1 h. Diaminobenzidine tetrahydrochloride was added for development for 10 min, followed by counterstaining with hematoxylin. Negative control slides omitting the primary antibody were included in all assays.

Immunostaining was reviewed using conventional light microscopy and scored by a board-certified gynecologic pathologist (PMF), by intensity and percentage of positive tumor cells. Staining intensity was categorized as 0, 1+ (light brown), 2+ (moderate brown) or 3+ (dark brown), and by percentage of positive tumor cells categorized as 0 for none, 1 for ≤10%, 2 for 11–25%, 3 for 25–50%, 4 for 51–75% and 5 for >75%. Cores were not evaluated if the core was lost, severely damaged, and/or did not have sufficient tumor cellularity. The reviewer was blinded to clinical data. Uterine cancer cases were excluded from statistical analysis if less than three cores were available for analysis secondary to inadequate cancer tissue or poor quality of the specimen. Tumor staining for nestin was very consistent across individual full tissue sections and across triplicate cores for individual patients. For purposes of primary analysis, nestin expression was categorized as low (≤10% of tumor cells with positive staining) versus (vs.) high (>10% of