Review

Uterine Malignant Mixed Mullerian Tumor: Review of Recent Literature

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Uterine Malignant Mixed Mullerian Tumor (MMMT) is an uncommon neoplasm with aggressive clinical course. It shares similar etiology, clinical and pathologic features of high-grade endometrial carcinoma. As such, uterine MMMT has been recently regarded as high grade de-differentiated endometrial carcinoma. Histologically, uterine MMMT is composed of an admixture of malignant epithelial and mesenchymal components. Substantial immunohistochemical and molecular genetic evidence has suggested that these two components share the same cellular origin and the mesenchymal component might derive from the epithelial component via epithelial-mesenchymal transition. This article reviews the recent literature related to the pathogenesis, histopathology, diagnosis, and management of uterine MMMT.
[N A J Med Sci. 2017;10(3):110-115. **DOI:** *10.7156/najms.2017.1003110]*

Key Words: *malignant mixed mullerian tumor, MMMT, epithelial-mesenchymal transition, endometrial carcinoma*

INTRODUCTION

Malignant Mixed Mullerian Tumor (MMMT) is a highly aggressive neoplasm arising from the female reproductive organs, including the uterus, ovary, fallopian tube and vagina. Of these, uterine corpus involvement is the most common.[1] Rarely, it can arise from extragenital regions such as the peritoneum.[2]

The incidence of uterine MMMT is 2 per 100,000 women per year, accounting for about 2-5 % of all uterine malignancy. Uterine MMMT mostly affects postmenopausal women and is more prevalent in African American women.[3] Similar to high-grade endometrial carcinoma, the risk of developing uterine MMMT is increased in the setting of increased estrogen levels, nulliparity,obesity, high expression of insulin and insulin-like growth factors (IGFs) and inflammatory markers (interleukins). The risk is decreased by oral contraceptive pill usage.[3-5] Also, uterine MMMTs have been reported to occur after long-term tamoxifen therapy for breast cancer.[6-9] Germline BRCA1 mutation and prior pelvic radiation also are identified as risk factors for uterine MMMT.[10] In general, there is etiological overlap between uterine MMMT and endometrioid carcinomas, suggesting similar biological mechanisms underlying tumorigenesis of these tumors.

PATHOGENESIS

Uterine MMMT is a biphasic neoplasm that is composed of malignant epithelial and mesenchymal components. The histopathogenesis has been debated whether the tumor arises from separate synchronous tumors to form a collision tumor (biclonal tumor theory), or the epithelial and mesenchymal components are from the common cellular origin and mesenchymal component derives from epithelial component via metaplasia (monoclonal tumor theory). Extensive immunohistochemical and molecular genetic studies have provided substantial evidence to support the monoclonal histogenesis of uterine MMMT. The immunoreactivity of p53 and p16 is concordantly detected in the epithelial and mesenchymal components in the majority of cases of uterine MMMTs.[11,12] Topographic genotyping in the subset of p53 immunoreactive tumors reveals the identical point mutations of p53 in both components.[11] Molecular profiling of uterine MMMT reveals the same alterations of p53 and PI3K pathway molecules in both epithelial and mesenchymal components, and in the primary tumor and metastatic sites.[13] In another wide genomic sequencing study of 42 cases of uterine and ovarian MMMTs, many similar mutations typical of uterine and ovarian epithelial cancers, such as TP53, PIK3CA, PPP2R1A, KRAS, PTEN, CHD4, and BCOR[14], were identified in both components as well.

It has been recently proposed that the mesenchymal component of uterine MMMT derives from endometrial carcinoma via epithelial–mesenchymal transition (EMT), a process in which epithelial cells lose polarity and cell to cell contact. Unlike metaplasia, EMT involves the formation of motile cells from parent epithelial cells that are not motile themselves. This process has been widely implicated in embryonal development and cancer metastasis. In cancers,

Received: 07/01/2017; Revised: 07/20/2017; Accepted: 07/22/2017
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malignant epithelial cells switch to a motile mesenchymal phenotype upon tumor microenvironment-dependent stimuli or by innate activation of EMT pathway.[15] Tumor cells thus have greater motility and invasion capacity for their dissemination and metastasis. Also, tumor cells become more resistant to apoptosis and therapeutic drugs. Interestingly, tumor cells that have acquired a mesenchymal phenotype can undergo the reverse mesenchymal–epithelial transition process, which allows them to reacquire an epithelial phenotype and its functionalities. EMT is, therefore, becoming a target of prime interest for anticancer therapy.[15] EMT is characterized by progressive loss of E-cadherin coupled with the expression of nonepithelial cadherins (N-cadherin and cadherin-11) and mesenchymal markers (vimentin, fibronectin, and others) in lung and breast cancers.[16] In the same genomic sequencing study of ovary and uterine MMMTs, an excess of mutations in genes encoding histone H2A and H2B are identified. Stable transgenic expression of mutant H2A and H2B in a uterine serous carcinoma cell line increases expression of markers of EMT as well as tumor migratory and invasive properties, suggesting their role in the sarcomatous change in MMMT.[14] EMT is also regulated by microRNAs (miRNAs), a class of small non-coding RNA molecules that functions in RNA silencing and post-transcriptional regulation of gene expression. Compared to endometrial carcinoma, serous carcinoma, and endometrial stroma sarcoma, uterine MMMT is featured by aberrant regulation of mir-200 and mir-205. The downregulation of these miRNAs is possibly mediated by epigenetic methylation.[17] In addition, miR-200C inhibits the growth of uterine MMMT cells by driving the mesenchymal-to-epithelial transition. Regulation of miRNAs could be a potential treatment approach for patients with MMMT.[18]

The epithelial component of MMMT is believed to be the driving force of tumor progression. Interestingly, uterine MMMT exhibits heterogeneous molecular features that resemble the heterogeneity seen in endometrial carcinomas, with some showing endometrioid carcinoma-like mutations and some showing serous carcinoma-like mutation profiles.[13] Patients with serous-like tumors present more frequently with the advanced-stage disease compared to those with endometrioid-like tumors.[13] Based on the type of sarcomatous differentiation, the mesenchymal component can be homologous that is native to the uterus or heterologous that is foreign to the uterus. The presence of rhabdomyoblastic component has been associated with advanced stage and poorer prognosis in some studies.[19,20] Similar to endometrial carcinoma, uterine MMMT may represent two categories, low-grade and high-grade, based on their genetic alterations, histological subtypes, and clinical courses. The carcinomatous component of uterine MMMT may be in a more primitive stage which allows a subset of carcinomatous cells to transform to their distinctive mesenchymal phenotype. Indeed, some studies have demonstrated the presence of cancer stem cells in uterine MMMT. The CD133-positive stem cells that are separated from uterine MMMT are capable of differentiating into mesenchymal cells and are more resistant to cisplatin/paclitaxel-induced cytotoxicity when compared to CD133-negative cells. Moreover, the strong CD133 expression in both epithelial and mesenchymal elements in primary tumor is associated with a significantly poorer prognosis.[22]

CLINICAL FEATURES

Patients with uterine MMMTs usually present with postmenopausal bleeding, abdominal pain, and uterine enlargement. Ultrasound often shows an endometrial mass or a mass prolapsing from the cervix. Extrauterine disease is present in up to 60% patients with uterine MMMTs. Tumor recurrence is more than 50% despite surgery and adjuvant therapy. It is important to utilize imaging with CT and MRI before surgery to help guide patient counseling and surgical planning. Serum CA 125 level is another useful marker to clinical evaluation. Elevated serum CA125 is associated with the presence of extrauterine disease, serous epithelial component and deep myometrial invasion.[21]

PATHOLOGY

Grossly, uterine MMMTs are soft, friable, necrotic and hemorrhagic tumors which fill the endometrial cavity and may protrude through the cervix. The tumors often have cystic and solid areas with polypoid features. They often have deep myometrial invasion and cervical involvement at presentation.

Microscopically, MMMTs are bi-phasic tumors composed of both malignant epithelial and mesenchymal components with a sharp demarcation. The epithelial component is often high grade. It can be composed of serous (66%), endometrioid (42%), clear cell and undifferentiated carcinoma in order of frequency.[22,23,24] In some cases, a hybrid morphology with serous and endometrioid carcinoma or squamous differentiation is present, which may be a clue to the diagnosis. Homologous mesenchymal component (**Figure 1A**) includes endometrial stromal sarcoma, fibrosarcoma or leiomyosarcoma. The heterologous mesenchymal component includes rhabdomyosarcoma (**Figure 1B**), chondrosarcoma (**Figure 1C**), osteosarcoma or liposarcoma. Rarely, a component of primitive neuroectodermal tumor (PNET)(Fig. 1D) or neuroendocrine, choriocarcinoma and melanocytic differentiation can be seen.[24]

IMMUNOHISTOCHEMISTRY

By immunohistochemistry, the epithelial component is usually strongly and diffusely positive for pancytokeratin, epithelial membrane antigen (EMA), and variably immunoreactive for vimentin. ER and PR are mainly expressed in the epithelial component in 22% and 11% of uterine MMMTs, respectively (**Figure 2A** and **Figure 2B**).[25,26] PAX8 is almost ubiquitously and extensively expressed in the epithelial component of uterine MMMTs (97%), but much less common in sarcomatous and undifferentiated components is much less common. This makes PAX8 a useful makes PAX8 a useful marker for the diagnosis of carcinomatous metastases of uterine MMMTs to extrauterine sites.[27] p16 and p53 can be seen in both epithelial and mesenchymal components and are more frequently associated with serous epithelial histology.

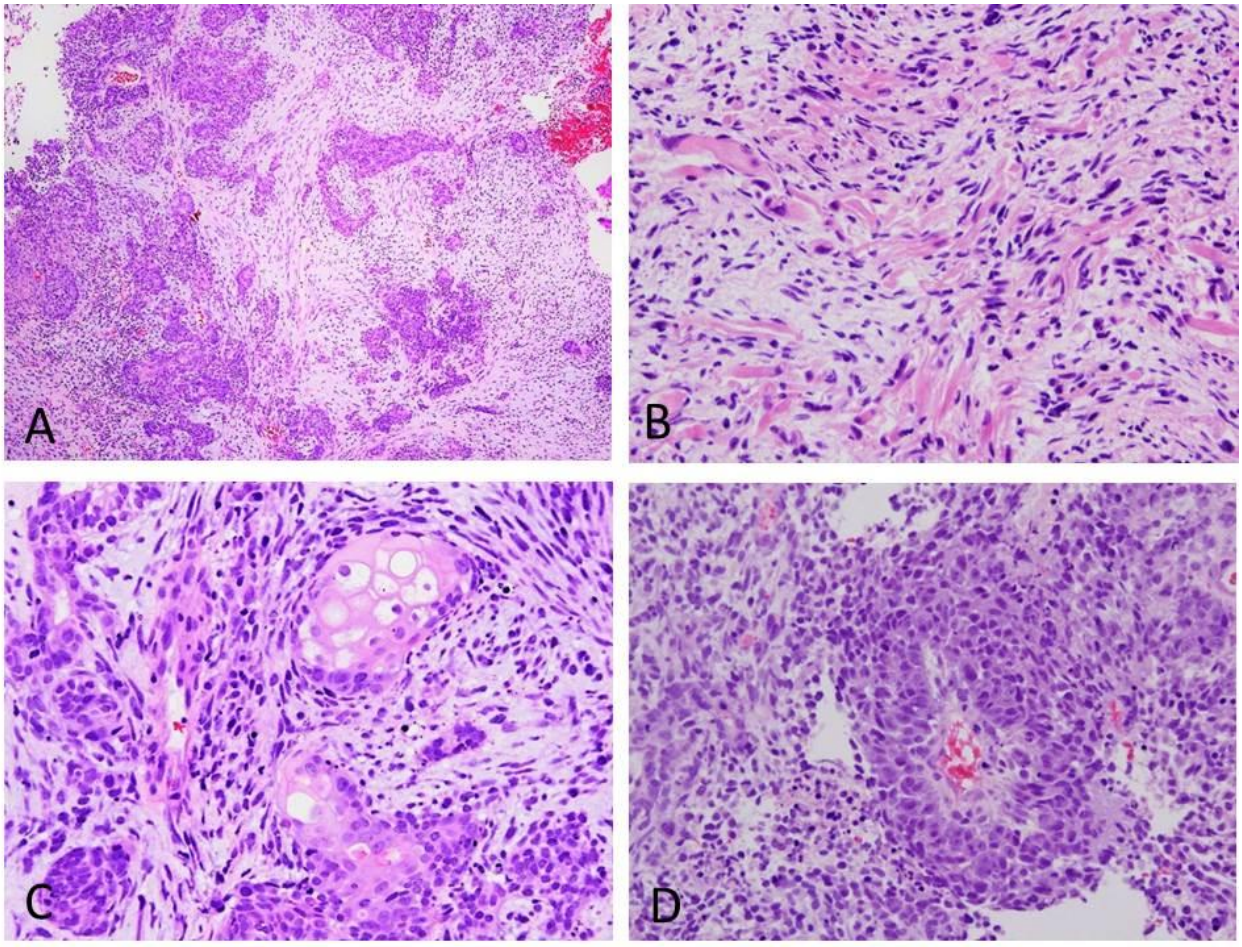

Figure 1. Morphological variations of sarcomatous component of MMMT. The mesenchymal component can be (**A**) homologous sarcoma not otherwise specified, (**B**) heterologous sarcoma with rhabdomyoblastic feature, (**C**) chondrosarcoma, and (**D**) primitive neuroectodermal tumor.

The mesenchymal component is usually diffusely positive for vimentin, patchy positive for cytokeratins and EMA. The homologous sarcomatous component frequently expresses CD10 (**Figure 2C**) and CD34. The heterologous elements can be highlighted by the expression of antibodies specific to type of sarcomatous differentiation. When the mesenchymal component displays histologic features of rhabdomyosarcoma, desmin and myogenin can be used. Myoglobin is more specific but less sensitive to stain rhabdomyoblasts. S100 is used for identification of chondroid or lipomatous differentiation. Synaptophysin (**Figure 2D**), chromogranin and CD56 are markers for neuroendocrine differentiation. SATB2 is a novel marker of osteoblastic differentiation and is highly sensitive for identification of osteosarcomatous component in uterine MMMT.[28]

DIFFERENTIAL DIAGNOSIS

The histopathologic hallmark of uterine MMMT is the admixture of high-grade epithelial and mesenchymal components. The monophasic tumor should not be diagnosed as uterine MMMT, although it is acknowledged that either the mesenchymal or epithelial component of uterine MMMT might predominate in small samples such as biopsies and scant curettage specimens. Extensive sampling of specimens is warranted for appropriate interpretation. The differential diagnosis of uterine MMMT consists predominantly of tumors that also have a biphasic appearance as listed below:

A. Mullerian adenosarcoma is a biphasic tumor composed of benign epithelium and malignant stroma. It is conceptually distinguisded from uterine MMMT by its benign epithelium. However, some uterine MMMTs might also arise from adenosarcomas. Evidence has shown that one-third of uterine MMMTs contain zones that closely resemble adenosarcoma. Histologically, adenosarcoma has a characteristic leaflike cleft architecture which resembles a phyllodes tumor of the breast. The epithelium is typically bland endometrioid with frequent squamous or mucinous metaplasia. The mesenchymal component is usually a low-grade spindle cell sarcoma or low-grade endometrial stromal sarcoma. Periglandular stromal

hypercellularity, also called periglandular cuffing, is also characteristic. This entity often shows immunoreactivity for ER and PR in both glandular and stromal components. However, expression of these markers is significantly decreased in adenosarcoma with sarcomatous overgrowth. CD10 can highlight the stroma and periglandular cuffing. Other mesenchymal markers, such as smooth muscle actin (SMA), desmin, and CD34, have variable positivity in the stromal component.[29]

B. Endometrioid carcinoma with spindle cell elements is another biphasic endometrial neoplasm that can mimic MMMT. In this tumor, the epithelial component is typically low-grade (grade 1 or 2) endometrioid carcinoma frequently showing squamous metaplasia. The distinctive fusiform spindle cell component sometimes is mitotically active, but not markedly atypical. The spindle cells are positive for cytokeratins, ER, PR, and show patchy p16 positivity with wild-type p53 expression pattern.[30] Endometrioid carcinoma with sex cord–like formations and hyalinization may display cords of epithelioid or spindle cells within a hyalinized stroma and, infrequently, osteoid or chondroid differentiation. These features raise concern for uterine MMMT, especially because of the "biphasic" appearance of these tumors. This tumor shows nuclear β-catenin expression, ER, PR, and patchy p16 positivity. It tends to present at a low stage and has a favorable prognosis.

C. Dedifferentiated endometrial carcinoma is characterized by low-grade (grade 1 or 2) endometrioid carcinoma adjacent to the undifferentiated component. The latter is composed of small, round cells of uniform size in a solid sheet, instead of spindle-shaped or pleomorphic cells. Unlike the differentiated component, the undifferentiated component tends to be negative for PAX8, ER, and PR with variable expression of cytokeratins. They can be associated with microsatellite instability.[30] Given their biphasic appearance and the presence of focal nuclear pleomorphism in some cases, dedifferentiated carcinomas may be misdiagnosed as MMMTs. However, these tumors tend to occur at a younger age, and the presence of a low-grade gland-forming endometrioid component and heterologous elements are clues to the right diagnosis.

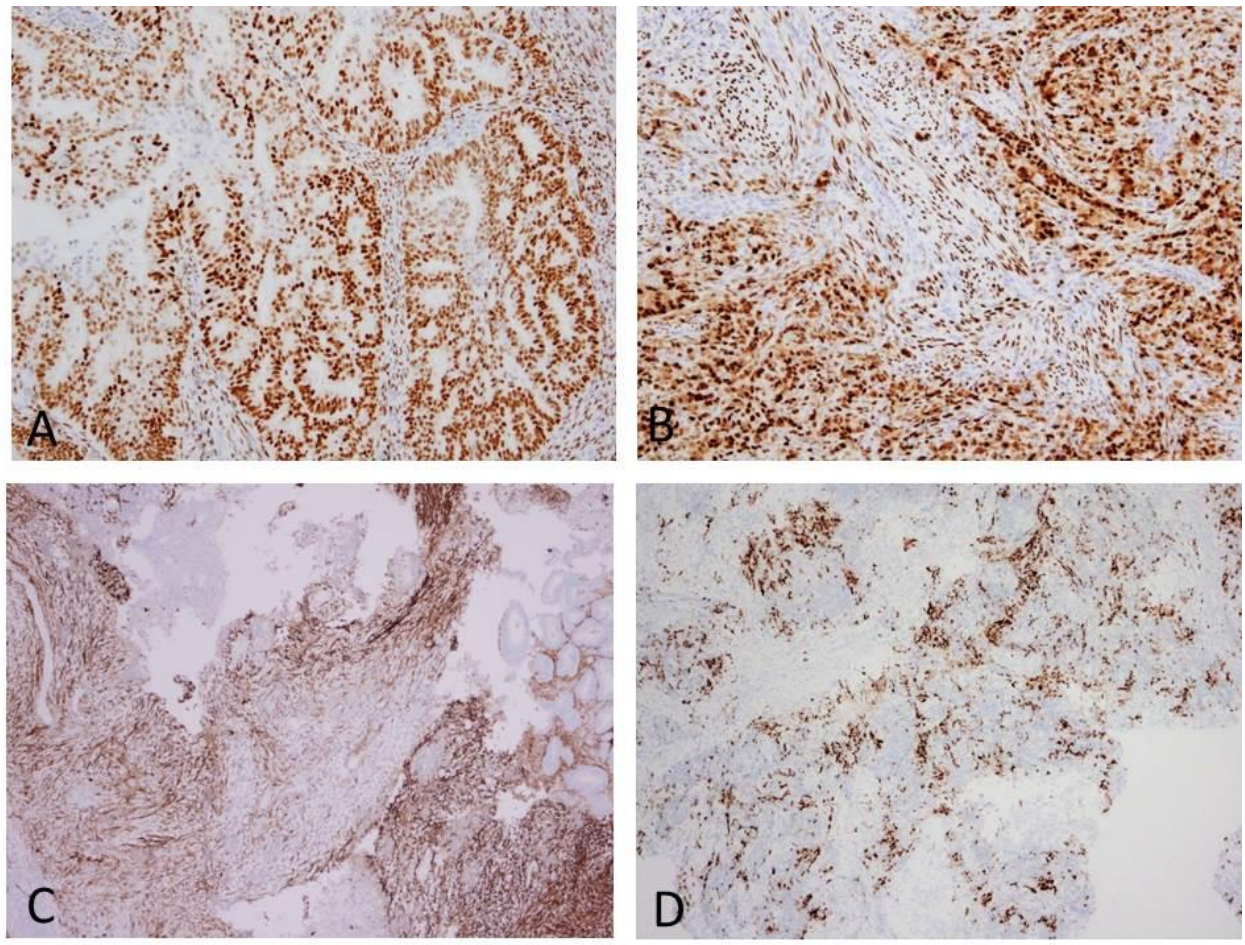

Figure 2. Immunohistochemical staining of uterine MMMT. The tumor is stained for (**A**) ER, (**B**) PR, (**C**) CD10, (**D**) synaptophysin.

TREATMENT

There are limited therapeutic options for patients with uterine MMMT. For patients with early stage of disease, complete surgical staging is the treatment of choice, including hysterectomy, bilateral salpingo-oophorectomy, and retroperitoneal lymph node dissection. Adjuvant chemotherapy in patients with early stage of the disease is associated with improved progression-free survival, but overall survival is improved only in the absence of lymphovascular invasion.[31] For patients with advanced stage of MMMTs who often have an extrauterine disease, cytoreductive surgery is recommended to improve the survival rate. In a multi-institutional retrospective study, 76% (170/225) patients with advanced stages received optimal cytoreductive surgery. Their median progression-free survival and overall survivals were 11.5 and 37.9 months versus 8.1 and 18 months for patients who received optimal and suboptimal cytoreductive surgery ($p< 0.01$), respectively.[32] Adjuvant chemotherapy is recommended in the advanced stage of uterine MMMT. The optimal chemotherapeutic regimen is still debated.

PROGNOSIS

Uterine MMMT is more aggressive than FIGO Grade 3 endometrioid, serous and clear cell carcinomas.[19,20] The 3-year and 5-year disease-free survival rate is 42% and 12-20% for women with uterine MMMT, respectively. Similar to other high-grade endometrial cancers, the metastasis of epithelial component is primarily via lymphatics and most deaths in patients are secondary to locally recurrent pelvic and abdominal disease. The factors associated with a good prognosis of uterine MMMT includes age below 40, white race, early stage of disease with a limited extent of the disease, absence of myometrial invasion or lymphovascular invasion, and negative peritoneal cytology. Also, p53 status has also been reported to associate with poorer prognosis. The survival time for patients with p53-positive uterine MMMTs is 3.56 years as opposed to 8.94 years in those with p53-negative cases, whereas overexpression of p16 and Mcl-1 is observed in patients with longer survival outcomes.[33]

CONCLUSION

Uterine MMMT is a relatively rare gynecological neoplasm. This tumor behaves similar as but more aggressive than high-grade endometrial carcinoma. The prognosis is very poor, and there are limited therapeutic options. Recent studies regarding the EMT process and cancer stem cells have revealed some of the underlying molecular and cellular mechanisms. Related clinical trials should be done in the future to develop new therapeutic strategies in this highly aggressive tumor.

CONFLICT OF INTEREST

There is no conflict of interest to declare.

ACKNOWLEDGEMENTS

Authors like to express their thanks for the suggestions from Dr. John Tomazewski, Department of Pathology, University at Buffalo.

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