### Title

Serous carcinomatous component championed by heparin-binding EGF-like growth factor (HB-EGF) predisposing to metastasis and recurrence in stage I uterine malignant mixed mullerian tumor.




### Authors

Zhang, Lei
Shimizu, David
Killeen, Jeffrey L
et al.



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# Serous Carcinoma component championed by Heparin Binding-EGF Like Growth Factor (HB-EGF) Predisposing to Metastasis and Recurrence in Stage I Uterine Malignant Mixed Mullerian Tumor

**Lei Zhang, MD PhD**[1,2,*], **David Shimizu, MD**[1,3], **Jeffrey L. Killeen, MD**[1,4], **Stacey A. Honda, MD PhD**[1,5], **Di Lu, MD**[6], **Alexander Stanoyevitch, PhD**[7], **Fritz Lin, MD**[6], **Beverly Wang, MD**[6], **Edwin S Monuki, MD PhD**[6], and **Michele Carbone, MD PhD**[1,8]

[1]The Department of Pathology, John A. Burn School of Medicine, the University of Hawaii, Honolulu, HI 96813

[2]The clinical informatics fellowship program, University of California at Los Angeles, Los Angeles, CA 90095

[3]The Department of Pathology, the Queen's Medical Center, Honolulu, HI 96813

[4]The Department of Pathology, Kapi'olani Medical Center for Women and Children, Honolulu, HI 96826

[5]Department of Pathology, Kaiser Permanent, Honolulu, HI 96819

[6]The Department of Pathology, University of California at Irvine, Irvine, CA 92868

[7]Department of Mathematics, California State University-Dominguez Hills

[8]Cancer Center, University of Hawaii, Honolulu, HI 96813

## Abstract

The stage I uterine Malignant Mixed Mullerian Tumor (MMMT) shows different potential for progression. We reason that MMMTs with high grade carcinomatous component and positivity for HB-EGF are prone to recurrence/metastasis in the early stage. A retrospective clinical and histopathologic review with immunohistochemical staining for HB-EGF, EGFR, and integrin-α5 was performed for 62 surgically staged MMMT cases. Recurrence/metastasis (RM) is 6/18(33%) in stage I diseases. Of all the clinicopathologic variables and biomarkers analyzed for stage I MMMT, serous carcinomatous component [83% (5/6) versus 17% (1/12), $p$=0.0015] and HB-EGF

[*]**Corresponding author**: Lei Zhang MD PhD, lei_248@hotmail.com.



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**Competing interest**

MC has pending patent applications on BAP1 and HMGB1 and provides consultation for mesothelioma and expertise and diagnosis. The other authors declare no conflict of interest.

expression [100% (6/6) versus 50% (6/12), *p*=0.0339] are significantly different between group with RM and without RM. The presence of serous carcinoma in all stages is: 83% (5/6) in stage I with RM, 8% (1/12) in stage I without RM, 20% (1/5) in stage II, 36.4% (8/22) in stage III and 64.7% (11/17) in stage IV; this is paralleled by HB-EGF expression of 100% (6/6), 50% (6/12), 40% (2/5), 50% (11/22) and 71% (12/17) with a correlation coefficient r=0.9131(p=0.027). HB-EGF and integrin-α5 are highly expressed in MMMTs bearing serous carcinoma component, compared to endometrioid and unclassifiable/miscellaneous subtypes (84.6%/47.6%/33.3%, p=0.025 for HB-EGF; and 61.5%/42.9%/20.0%, p=0.021 for integrin-α5). The EGFR positivity is comparable among the three subtypes (48.1%, 47.6% and 26.7%, p= 0.326). This study indicates that serous carcinomatous component championed by expression of HB-EGF predisposes to recurrence/metastasis in stage I MMMT. This process might involve integrin-α5 and does not seem to require overexpression of EGFR. Further study is required.

### Keywords

Malignant Mixed Mullerian Tumor (MMMT); serous carcinoma; AJCC stage; tissue microarrays (TMA); Heparin binding-epidermal growth factor like growth factor (HB-EGF); Epidermal growth factor receptor (EGFR); integrin-α5

## Background

Malignant Mixed Mullerian Tumor (MMMT) is a biphasic tumor derived from Mullerian (paramesonephric) duct accounting for less than 5% of gynecologic malignancy. MMMT is most often identified in the uterus, less commonly in the ovary, fallopian tube and peritoneum. It has both carcinoma and sarcoma components. More and more evidence suggests that those tumors are of epithelial origin demonstrating epithelial-mesenchymal transition (EMT). [1,2,3].

MMMT carries a poorer prognosis than other high grade endometrial carcinomas [3–5], especially for those with early stage diseases [5,6]. Even completely staged early (stage I, or I & II) MMMT cases had a significant shorter median overall survival and disease free survival than matched high grade endometrial carcinoma controls [5,6]. There was a statistically significant difference in time until recurrence between women with stage I MMMT and those with stage I high grade endometrial carcinomas, 25 months (95% CI, 14-not reached) versus not reached [6]. One study showed a worse outcome for uterine MMMT versus uterine serous carcinoma in 5-year actuarial rates of recurrence [45% (CI. 31–59%) vs. 17% (CI. 10–25%), p<0.001], disease-related mortality [30% (16–44%) vs. 11% (5–17%), p=0.016], and all-cause mortality [34% (CI. 20–48%) vs. 12% (CI. 6–18%), p=0.007]; however, this difference is not significant in a subgroup of MMMT patients able to receive adjuvant intravaginal radiotherapy and chemotherapy [7]. The high risk of recurrence/metastasis and promise of adjuvant therapy in early stage MMMT have prompted research to identify parameters responsible for this progression. Early studies have suggested a heterologous sarcomatous element[6], histology of the carcinomatous component[8], lymphovascular invasion, size of tumor, depth of myometrium invasion, extent of sarcoma and peritoneal cytology findings [9] as adverse prognostic factors. Those factors showed

variable predictive powers for prognosis. We hypothesize that those variables are correlated and are under a canopy of a key histopathologic character. Considering of MMMT as a carcinoma with sarcomatous metaplasia, the carcinomatous component is likely to be the driving force. Heparin binding-epidermal growth factors like growth factor (HB-EGF) are secretary ligand functioning in epithelial-mesenchymal transition, tumor growth and metastasis in autocrine, juxtacrine and paracrine patterns [10–15]. They have been shown to bind to and activate epidermal growth factor receptors EGFR/ErbB1, promoting proliferation and survival through MAPK and PI3K pathways [11,12]. Binding of HB-EGF to ErbB4/HER4 induces chemotaxis but not proliferation [16]. The soluble form of HB-EGF generated through a process of ectodomain shedding through matrix metalloproteinases and disintegrin, might decrease the expression of E-cadherin promoting epithelial-mesenchymal transition [14]. Loss of E-cadherin up-regulates integrin facilitating cell adhesion and spreading in ovarian cancer [15]. HB-EGF has also been shown to upregulate integrin α5β1 [17]. Lastly, HB-EGF mRNA expression has been detected in MMMT and is association with advanced stages in our previous study [18]. Therefore it is tempting to explore potential roles of HB-EGF, integrin- α5 and EGFR in recurrence and metastasis.

Integrins are heterodimeric transmembrane receptor proteins comprised of various combinations of an α- and a β- subunit. Fibronectin binding to α5β1-integrin led to a direct association of integrin-α5 with the receptor tyrosine kinase c-met, subsequently activating Src and focal adhesion kinase (FAK), contributing to invasion and metastasis [19]. It is also one of the potential HB-EGF downstream targets as aforementioned [17].

EGFR expression has been detected in 45%, 50% and 82% of MMMT in different studies [20, 21, 22]. It falls into the wide range of 34–67% for endometrial carcinoma and 30–98% for ovarian cancer [23]. The correlation of EGFR expression to any clinical phenotype of gynecologic tumor is controversial. However, EGFR is one of the receptors which HB-EGF can bind to. We are interested in knowing whether there is differential expression of EGFR in different stages of MMMT.

We hypothesize that MMMTs with high grade carcinomatous component and positivity for HB-EGF are prone to recurrence/metastasis in the early stage. Expression of integrin-α5 might be related to activation of HB-EGF. The requirement of EGFR for HB-EGF activation is intriguing and needs to be explored.

We consider that the prognostic histopathologic parameter(s) and biomarker(s) predispose to occult metastasis in early stage, and they are predominately present in advanced diseases. Therefore, we identify the susceptible histologic feature and biomarker through comparison of two groups of stage I diseases which are negative or positive for recurrence/metastasis (RM); and evaluate them again in advanced diseases.

## Methods

### Case collection

After approval by the Institutional Review Boards (IRBs) of Queen's Medical Center, the Hawaii Pacific Health, and Kaiser Permanent at Hawaii, an archival search at the

departments of pathology using terms of “carcinosarcoma” or “Malignant Mixed Mullerian Tumor” over a period of 11 years (from 2000 to 2011) was performed. The search yielded 90 surgical and cytology gynecologic specimens from 73 patients. To be included in this study, each patient had to have a hysterectomy and a bilateral salpingo-oophorectomy with slides and paraffin embedded formalin fixed blocks available. MMMTs other than uterus origin were excluded. Two uterine tumors with no consensus diagnosis of MMMT showing indetermined demarcation of epithelial and mesenchymal components were also excluded. A total of 76 specimens from 62 patients, e.g., a final of 62 cases were included in this study. No neoadjuvant therapy was known for those patients. The slides were reviewed by at least two pathologists from the authors (at least one reviewer having over 20 years of gynecological subspecialty experience) and clinical information was retrieved from electronic medical records. All study cases were annotated with available clinical information in a manner that protected patient privacy.

## Pathologic diagnosis and staging

The pathologic diagnosis of MMMT meets the criterion of “admixture of high grade epithelium and mesenchyme” [1]. For carcinomatous components of MMMT, a tumor was classified as serous as long as it contained prototypic papillary and slit like space, lined by dyshesive cells showing budding and tufting with ruffled lumen and striking nuclear atypia [1،24]; recognized as endometrioid if it contained pure endometrioid carcinoma showing villoglandular architecture with smooth lumen, usually grade 2 out of 3 nuclear atypia, possible presence of seamless transition to solid area without any other carcinoma subtype [1،6،24]; and unclassifiable/miscellaneous if it contained either clear cell carcinoma or all other unclassfiable components. For sarcomatous components of MMMT, a tumor is grouped as homologous if it contains a high-grade, non-specific sarcoma [1]; or heterologous if it has rhabdomyosarcoma, chondrosarcoma, or osteosarcoma elements [1]. The percentage of sarcomatous elements was estimated under microscope. Staging follows TNM and FIGO classification for uterine carcinoma in AJCC, $7^{th}$ edition [25]. The definitions of T categories correspond to the stages accepted by FIGO. Stage I uterine cancer is the disease confined to the corpus uterine with T1a referring to tumor limited to endometrium or invading less than half of myometrium and T1b tumor invading one half or more of myometrium [25]. Recurrence/metastasis (RM) is defined as a newly identified tumor localized within the pelvis (recurrence) or outside the pelvis including intraperitoneal disease or distant organ involvement (metastasis) months after primary surgery of hysterectomy and salpingo-oophorectomy.

## Tissue Microarray (TMA) Construction

Morphologically representative areas of the epithelial and mesenchymal tumor components were marked on hematoxylin and eosin (HE) stained slides. A total of 274 cores (2 mm in size each) were taken following reviewing of 1124 slides, and arrayed on a recipient paraffin block using a tissue microarrayer. The TMA foci for each case are $6 \pm 2.3$.

## Immunohistochemistry (IHC)

The antibodies used are: human HB-EGF affinity purified goat polyclonal antibody, R&D, AF-259-NA, 1:100 dilution; Integrin α5 rabbit polyclonal antibody (H-104), Santa Cruz,

SC-10729, 1:100 dilution; EGFR mouse monoclonal antibody, prediluted clone 31G7, Zymed/Invitrogen, 08–1205. All the IHC stains were performed at a clinical immunohistochemistry laboratory using the Bond polymer detection system in Leica Bondmax autostainer. Briefly, the TMA blocks were sectioned at 5μm, deparaffinized and incubated with primary antibodies following antigen retrieval. Positive and rabbit IgG negative controls were included for each run and each slide.

The positive control for HB-EGF and integrin-α5 is placenta tissue. The negative controls for both markers are normal postmenopausal endometrium, normal ovary and fallopian tube. Squamous cell carcinoma of head and neck serves as positive control for EGFR.

The HB-EGF and integrin-α5 both show cytoplasmic staining with membrane accentuation. The IHC stain for both markers is evaluated by intensity and extent and reported as calculated H-score. The extent is graded as 0 (<5% tumor cell staining), 1 (5–50% of tumor cells staining) and 2 (>50% of tumor cells staining). The intensity is scored as 0 (no stain), 1 (weak stain) and 2 (strong stain). The H-score is the product of intensity and extent, and is graded as 0, 1+ (H-score value of 1), 2+ (H-score value of 2) and 3+ (H-score value of 4). EGFR shows membrane stain with some cytoplasmic expression. No stain, only cytoplasmic stain or weak membrane stain less than 5% is considered as negative. Weak membrane stain greater than 5% is 1+, and moderate to strong membrane stain is 2+.

### Statistical analysis

Clinicopathological features were compared using a t-test (continuous data: depth of invasion, tumor size and age) and Fisher's exact test (categorical data). Analysis of association of serous carcinoma component with heterologous sarcoma element, as well as association of biomarker expression and different subtypes of MMMT is performed using a chi-squared test. The correlation between MMMT histologic subtypes and biomarkers, as well as association among biomarkers is estimated by Pearson's correlation coefficient. A *P* value equal and/or less than 0.05 (P<0.05) was considered as statistically significant. The correlation must be 0.5 or above in either direction to be considered significant.

## Results

### Histologic subtype and prognosis

**I. Parameters associated with recurrence/metastasis in stage I MMMT—**In our stage I MMMT, 72% (13/18) of cases have had pelvic lymph node dissection and are considered as completely staged by surgery. In addition, nine cases which have had lymph node dissection also had peritoneal washing. The three cases which have positive peritoneal cytology all have had lymph node staging. The only case with omentectomy has had peritoneal washing and lymph node dissection and both are negative (Table 1). In the current AJCC/FIGO system, peritoneal cytology is no longer used for uterus cancer staging, because when the only evidence of extrauterine spread is positive peritoneal cytology, the influence on outcome is unclear [25]. As a result, although the collection of cytology specimens is still suggested, a positive result does not upstage the cancer.

Comparison of stage I MMMT with or without RM (Table 1) shows a significant difference in epithelial histologic subtypes between these two groups. The serous carcinoma element is more frequently present in stage I MMMT with RM compared to that without RM (p=0.0015). Our data show a trend for heterologous sarcomatous subtype to have a higher presence in the RM group, although this trend is not statistically significant. The RM cases are all stage T1a. The depth of myometrium invasion is paradoxically more superficial or even absent in the group with RM compared to that without RM during initial staging. Similarly, the lymphovascular invasion is identified in 4/12(33%) of cases in stage I without RM but in none of the six cases with RM 0/6(0%). The differences in patient age, tumor size, percentage of sarcomatous components, procedure (lymph node dissection and peritoneal washing) frequency between the RM and non-RM groups are not statistically different. There is no significant difference in the numbers of blocks submitted for initial pathologic diagnosis between the two groups (p=0.315).

**II. Differential presence of histologic subtypes in all stages of MMMT—** Coincidence with the over presence of serous carcinomatous element in stage I MMMT with RM, serous carcinoma shows a trend of stepwise increase across the tumor stage (Figure 1A). Compared to endometrioid carcinoma, serous carcinoma is more frequently associated with heterologous element in MMMT all stages combined (p=0.0227) (suppl Table 1).

## Differential expression of biomarkers

**I. Expression of biomarkers in different stages and different histologic subtypes of MMMT—**The representative biomarker expression in a case of stage I MMMT, with serous carcinomatous and homologous sarcomatous elements is shown in Figure 2. HB-EGF is differentially expressed in stage I MMMT with recurrence/metastasis (p=0.0339, in Table 1). It is also significantly over expressed in MMMT with serous carcinomatous components compared to endometrioid, or unclassifiable/miscellaneous carcinomatous components (p=0.025, in Figure 1B). Furthermore, there is a high correlation of serous carcinoma subtype with HB-EGF expression in different stages (r=0.9131, in Table 3).

Integrin-α5, is also preferentially expressed in MMMT with serous carcinoma component (p=0.026, in Figure 1B) compared with other subtypes, although showing a borderline correlation coefficient (r=0.470) with serous carcinoma in different stages (Table 3). The EGFR positivity is comparable among the three subtypes (48.1%, 47.6% and 26.7%, p= 0.326 in Figure 1B).

The explanatory analysis of relationship of these three biomarkers across the stages shows correlation coefficients of 0.5289, 0.1079 and 0.0845 for HB-EGF/ integrin- α5 (low significance), HB-EGF /EGFR(non-significant) and EGFR/integrin- α5 (non-significant) respectively (suppl Table 3).

**II. Intensity and extent of immunohistochemical staining—**The expression of each biomarker is evaluated by intensity and extent based on immunohistochemical staining. Illustration of representative score is shown in suppl Figure 1. The immunohistochemical staining results are tabulated based on histologic subtype and stage (Table 2). There is no

significant H-score difference for positive HB-EGF, EGFR or integrin-α5 staining among serous, endometrioid and unclassifiable/miscellaneous subtypes (p>0.05, in Table 2).

**III. Expression of biomarkers in carcinomatous and sarcomatous components—**HB-EGF, EGFR and integrin-α5 are positive in 43, 26 and 29 cases (suppl Table 2). Carcinomatous and sarcomatous components correspond in 41/43(95%) of HB-EGF, 25/26 (96%) of EGFR, and 29/29 (100%) of integrin-α5 immunostaining (suppl Table 2). For HB-EGF, there are two cases with HB-EGF 1+ staining limited to carcinomatous elements with the sarcomatous component being negative. Those two cases are endometrioid MMMTs, stage III and stage IV. For EGFR, there are 5 cases with biomarker expression exclusively limited to one of the two tumor elements: two 1+ cases positive only for carcinoma component (stage I endometrioid MMMT with no RM, and stage IV serous MMMT); one 2+ case positive for carcinoma component only (stage IV serous MMMT); two 1+ cases with positivity limited to sarcoma (one stage III endometrioid MMMT, and one stage IV serous MMMT). The differences of HB-EGF, EGFR and integrin-α5 expression in carcinomatous and sarcomatous elements are not statistically significant, p=0.848, 0.863,1.000 respectively (suppl Table 2).

## Discussion

Of all the histopathologic parameters studied in our cohort, only the serous carcinomatous element appears to be most significant in predicting recurrence/metastasis and possibly survival in early stage MMMT (table 1). This is similar to Desai and Gagne's findings [7, 8] and is in agreement of the role of carcinoma component as the driving force for MMMT.

There is also an association of a serous carcinomatous component with heterologous sarcomatous element in MMMT all stages combined (p=0.0227) (suppl Table 1), although this association was not significant in stage I disease when case numbers are limited (Table 1). This seems to agree with another report which demonstrated the presence of heterologous sarcomatous elements as a powerful negative prognostic factor in surgical stage I uterine MMMT in a study of 42 stage I MMMT cases when the histologic subtype of carcinomatous component is categorized as endometrioid and nonendometrioid rather than a separate serous component [6].

It is likely that the carcinoma component is the driver of epithelial mesenchymal transition (EMT), which leads to lymphovascular invasion and distant metastasis [1–3]. Therefore, it is no surprise that the extent of sarcomatous element and the presence of lymphovascular invasion are both associated with poor prognosis in early stage MMMT.

Deep myometrium invasion has been shown to be a negative prognostic factor for early stage MMMT in one study [8]. In our cohort, only MMMT endometrioid subtype shows linear increase in depth of myometrium invasion with advance of stage; this invasion pattern does not apply to nonendometrioid subtypes (suppl Figure 1). The stage I MMMT with RM shows paradoxically more superficial myometrium invasion compared to stage I MMMT without RM (Table 1). Therefore, the depth of invasion may not be a reliable factor

predicting worse prognosis if the carcinomatous component is nonendometrioid type in MMMT. This might indicate different invasion pathway among MMMT subtypes.

There are only five stage II cases (one serous, one UM and three endometrioid subtype) in our cohort and none of them showed recurrence/metastasis. Stage I with deep myometrial invasion (IB) and stage II endometrial carcinoma(stromal invasion of cervix) seem to have similar survival outcomes in a recent validation study of the prognostic performance of the staging system [26]. Therefore, the absence of worse prognosis in stage II disease compared to stage I in our cohort could be due to either overlapping of outcome between stage IB and stage II, or small number of stage II cases in our study.

HB-EGF, integrin- α5 and EGFR all show the highest expression level in stage IV. However, integrin- α5 and EGFR show lower level of expression in stage III relative to stage II, although they are both elevated compared to stage I without RM. Fewer stage II cases could be one of the concerns. The other possibility is that stages I-III uterine MMMT are diseases limited in pelvis and stage IV extends beyond peritoneum [25]. Mesothelial cells produce HB-EGF in physiological condition [27] and for tumor spreading [28, 29]. This paracrine mechanism, e.g., HB-EGF secreted from reactive mesothelial cells facilitating tumor metastasis, might partly hold responsibility for higher level expression of HB-EGF and integrin- α5. There is no mesothelial lining towards the inner side of pelvis; this may explain why the difference of biomarker expression is small between stage II and III.

HB-EGF has been shown to be expressed in advanced ovarian cancer and adjacent stroma but not in normal ovarian tissue by immunohistochemistry [30]. Using the same antibody, we have demonstrated the association of HB-EGF expression with serous carcinomatous component and FIGO staging in MMMT. Integrin-α5 is not detected in normal cycling or postmenopausal endometrium [31, 32], but was shown to be expressed in endometrial cancer, especially serous carcinoma [32–34]. Expression of integrin-α5 in MMMT in our cohort also shows similar trend as HB-EGF. Integrin-α5, which is known playing a role in invasion and metastasis [17], is reported to be upregulated by HB-EGF [15]. Therefore, the parallel expression of HB-EGF and integrin-α5 is consistent with the concept that integrin-α5 is one of the downstream targets of HB-EGF, related to tumor progression.

Similar to other publications, we were able to show EGFR expression in 44% (32/72) of MMMT cases [20,21]. However, the EGFR expression is close to equal in different stages and MMMT subtypes in our cohort. Statistical analysis did not show significant correlation between HB-EGF/EGFR or EGFR/integrin-a5 either. In the scenario of HB-EGF overexpression, this seems to be conflicting to our expectation that HB-EGF is a ligand binding to EGFR family [9,10]. Possible explanations to this could be: 1) HB-EGF has low affinity but high potency towards EGFR [35]; therefore high level of EGFR expression is not required for HB-EGF binding and nuclear translocation. 2) EGFR functioning requires phosphorylation. The immunohistochemistry used in our study demonstrates the presence of EGFR (phosphorylation or not, mutated or not), and does not directly indicate the function of EGFR. 3) HB-EGF is also able to activate vascular endothelial growth factor (VEGF) to facilitate invasion and metastasis [12,13]. It is possible that HB-EGF binding to VEGF may bypass EGFR pathway and obviate the need for EGFR overexpression. Therefore, the

expression of EGFR may not be necessary for disease progression. This speculation needs to be tested.

The expression of HB-EGF is upregulated directly or indirectly by multiple transcriptional factors through an autocrine loop [10]. P53 is among the wide variety of HB-EGF transcription activator candidates [10]. *TP*53 mutation is identified as a frequent event in MMMT genome in a recent study [36]. Serous carcinoma frequently showed p53 abnormality compared to endometrioid adenocarcinoma or other high grade endometrioid cancer [1]. The transcriptional activation of p53 towards HB-EGF has been referred in MMMT.

We summarize the relations of histologic subtype of MMMT, early stage recurrence/metastasis and biomarker expressions in Figure 3.

## Conclusions

Serous carcinomatous element appears to be highly significant in predicting recurrence/metastasis in stage I MMMT with possible inference for survival. This disease progression seems to be paralleled by HB-EGF expression, and might be related to the function of integrin-α5. The EGFR overexpression does not seem to be necessary in this pathway. Further mechanism study is warranted.

## Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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## List of abbreviations

| | |
|---|---|
| **AJCC** | American Joint Committee on Cancer |
| **MMMT** | Malignant Mixed Mullerian Tumor |
| **HB-EGF** | Heparin binding-epidermal growth factor like growth factor |
| **EGFR** | Epidermal Growth Factor Receptor |
| **TMA** | Tissue MicroArray |
| **TNM** | Tumor-Nodes-Metastasis |

| | |
|---|---|
| **FIGO** | International Federation of Gynecology and Obstetrics |
| **RM** | Recurrence/metastasis |
| **UM** | Unclassifiable/miscellaneous |
| **IHC** | immunohistochemistry |
| **H&E** | hematoxylin and eosin |
| **EMT** | epithelial mesenchymal transition |
| **VEGF** | vascular endothelial growth factor |
| **IRB** | institutional review board |
| **CI** | confidence interval |

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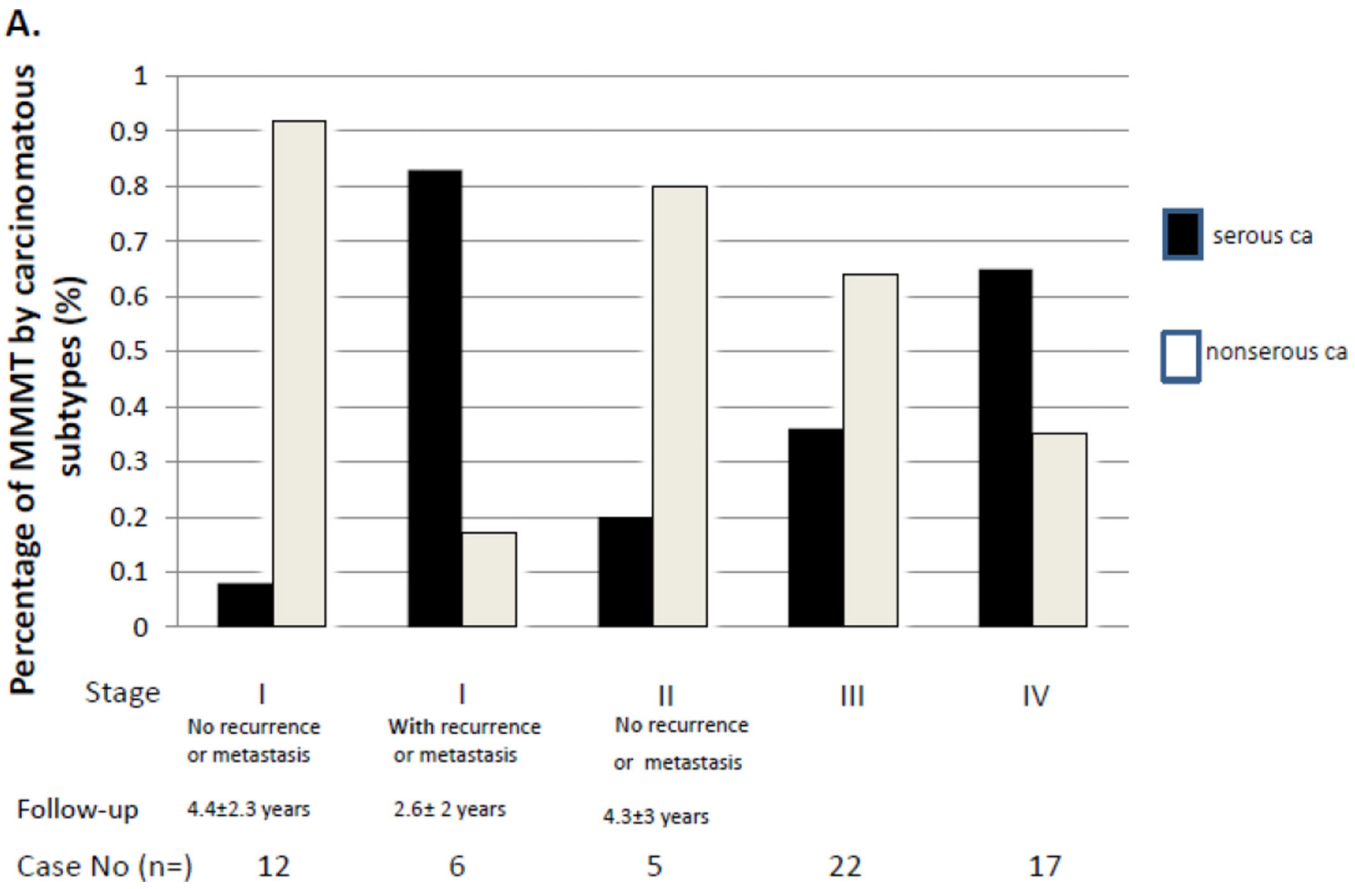


**Figure 1a**

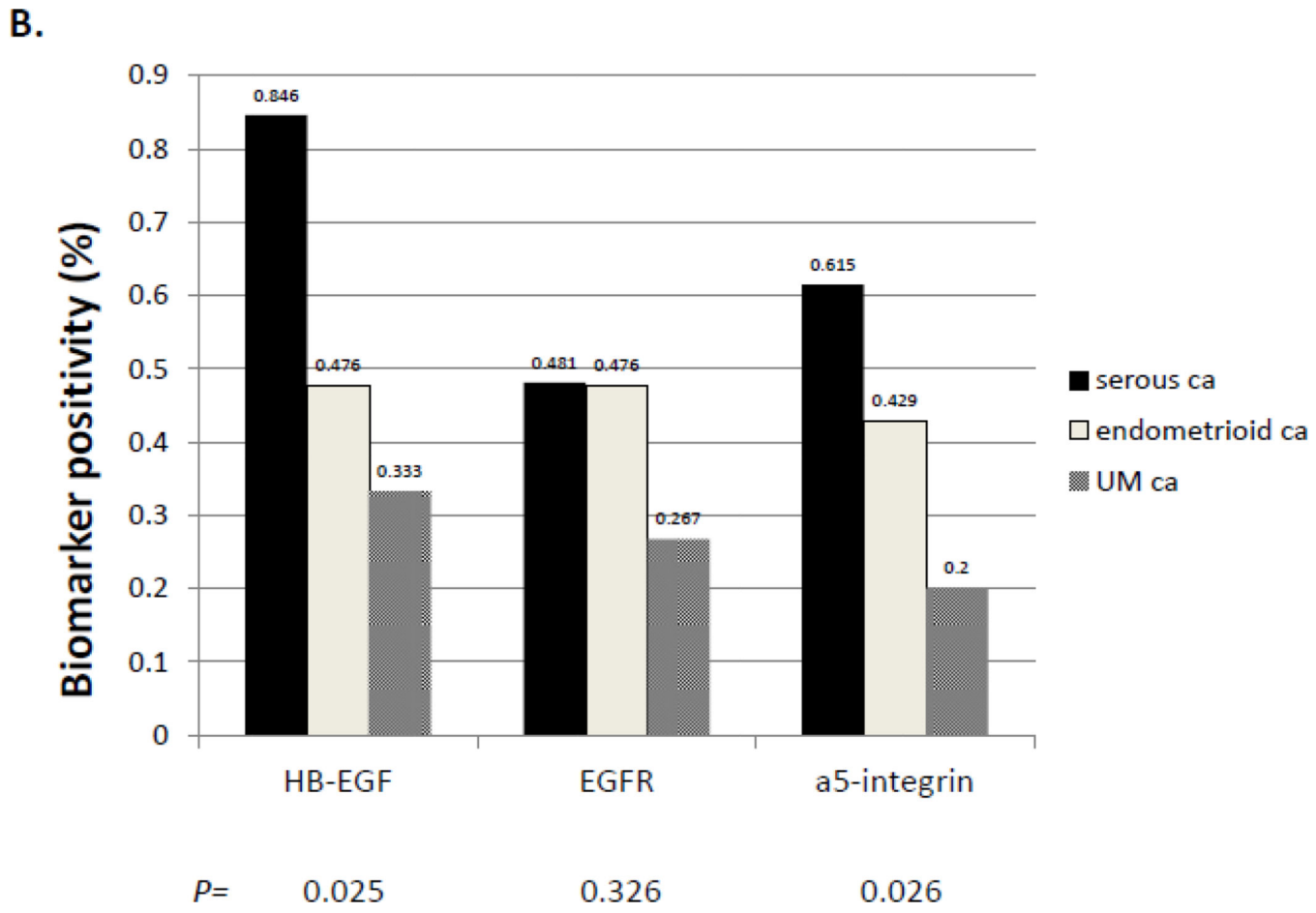


**Figure 1b**

**Figure 1.**

Various presence of carcinomatous subtypes of Malignant Mixed Mullerian Tumor (MMMT) across the stage (A) and biomarker expression in different carcinomatous subtypes (B)

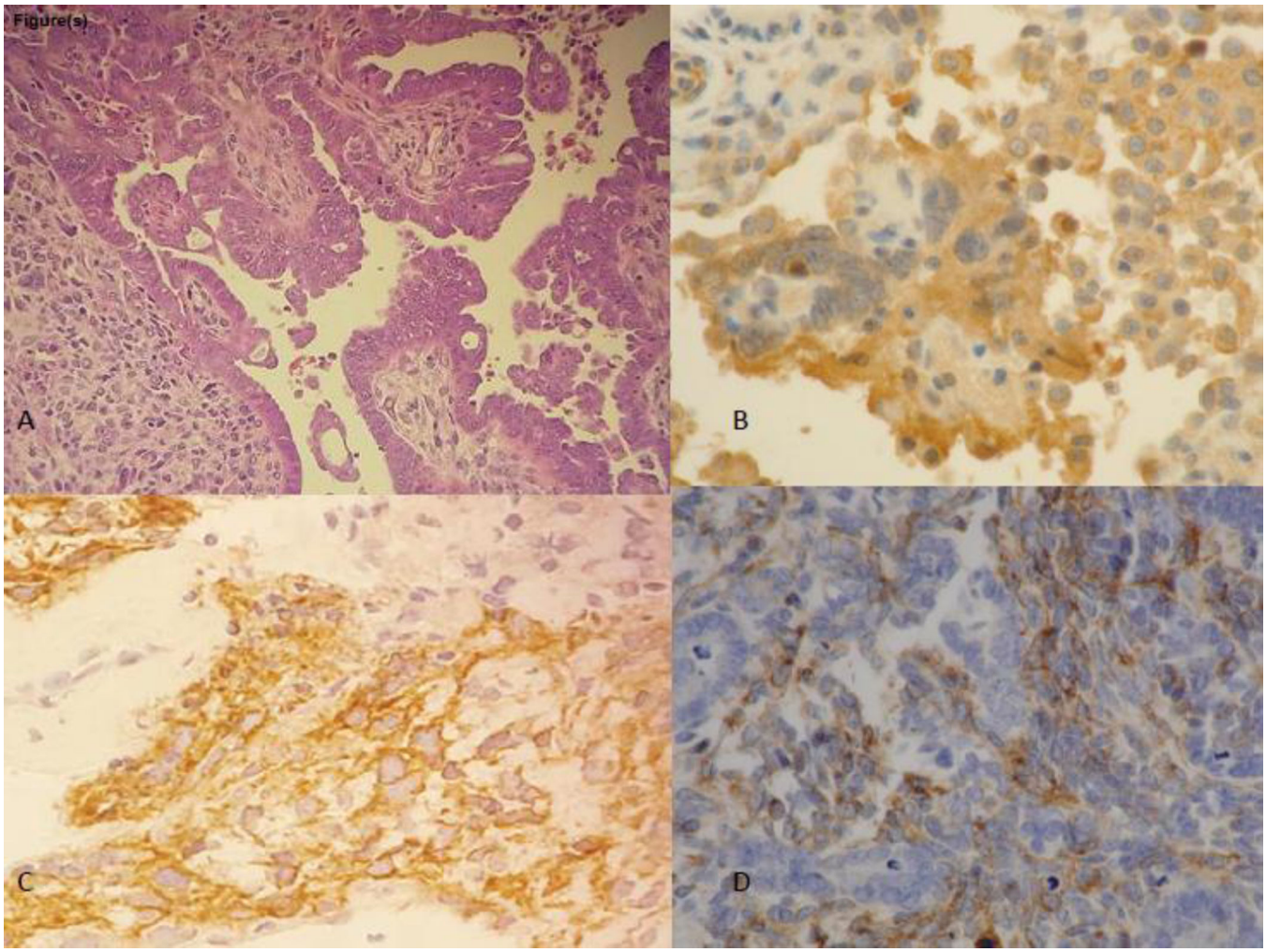


**Figure 2.**

Case illustration. A 65-year-old female presented with postmenopausal bleeding and intrauterine mass. The pathologic diagnosis is stage I uterine MMMT (tumor size 4.5 cm, no myometrium invasion) following a transabdominal hysterectomy and bilateral salpingo-oophorectomy. The patient was found to have vaginal recurrence in two months, and liver and lung metastasis in 3 months after surgery. A. H&E, MMMT with serous carcinoma and high grade homologous sarcoma; B-D, Immunohistochemistry: B. HB-EGF 2+; C. EGFR 2+; D. Integrin-α5 1+.

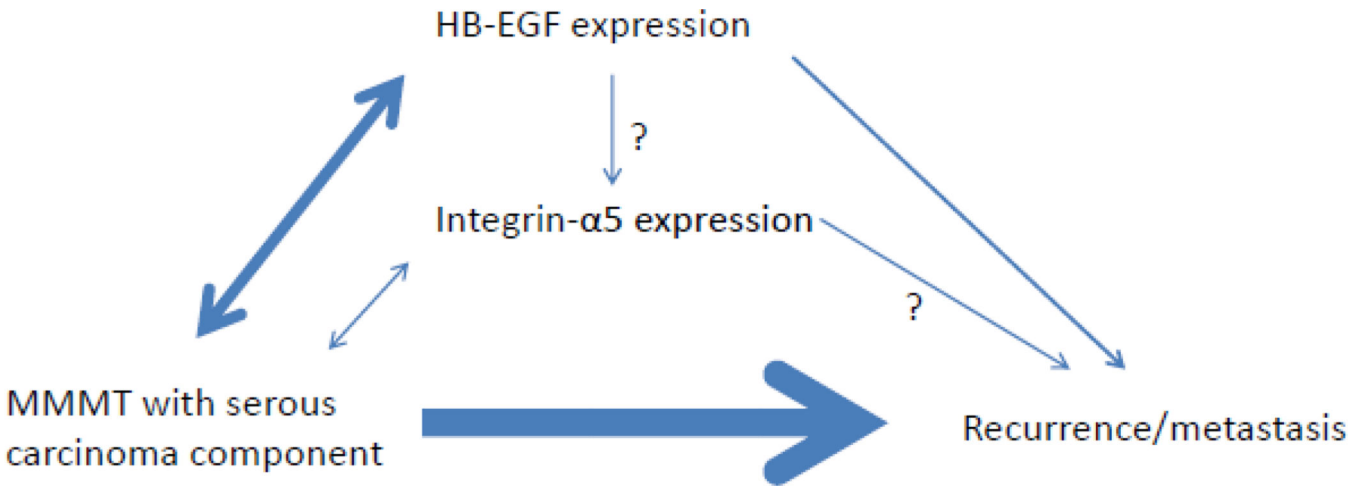


**Figure 3.**
Proposed relations among serous carcinoma, biomarker expression and recurrence/metastasis in Malignant Mixed Mullerian Tumor (MMMT)

## Table 1

Univariate analysis of clinicopathologic factors for recurrence/metastasis in stage I MMMT (t-test and Fisher's exact test)

| | Recurrence/metastasis | No recurrence/metastasis | p |
|---|---|---|---|
| Case number (n=) | 6 | 12 | |
| Age (mean±SD) | 68.2 ± 9.4 | 62.5 ± 13.3 | 0.1970 |
| **Surgical staging** | | | |
| - TAHBSO with pelvic lymph node dissection (n, %) | 5 (83 %) | 8 (67%) | 0.4390 |
| - TAHBSO with omentectomy (n, %) | 1 (17%) * | 0 (0%) | 0.2300 |
| - peritoneal washing performed (n, %) | 3 (50%) | 6 (50%) | 0.6900 |
| • • positive | 2 (67%) | 1 (17%) | 0.1300 |
| **Pathologic staging** | | | |
| Tumor size (mean ± SD) | 5.50 ± 3.28 | 6.13 ± 2.37 | 0.3220 |
| Depth of myometrium invasion (%, SD) | 9.0% ± 16% | 29.8% ± 33% | 0.0800 |
| Lymphovascular invasion (n, %) | 0 (0%) | 4 (33%) | 0.1620 |
| Stage IA (T1a) | 6 (100%) | 8 (68%) | 0.1100 |
| Stage IB (T1b) | 0 | 4 | |
| **Histology** | | | |
| - type of carcinomatous component | 0 | 0 | 0 |
| • • serous carcinoma (n, %) | 5 (83%) | 1 (17%) | 0.0015 |
| • • endometrioid carcinoma (n, %) | 1 (14%) | 6 (50%) | 0.1700 |
| • • unclassifiable/ miscellaneous (n, %) | 0 (0%) | 5 (100%) | 0.0630 |
| - type of sarcomatous component | 0 | 0 | 0 |
| • • heterologous (n, %) | 3 (50%) | 4 (33%) | 0.4900 |
| • • homologous (n, %) | 3 (50%) | 8 (67%) | 0.4900 |
| - Percentage of sarcomatous component | 0 | 1 | 1 |
| 0 • <50% (n, %) | 3 (50%) | 6 (50%) | 1.0000 |
| 0 • >50% (n, %) | 3 (50%) | 6 (50%) | 1.0000 |
| Extent of tissue examined | | | |
| - • number of blocks examined | 22.08 ± 8.02 | 24 ± 6.78 | 0.3150 |
| **Outcome** | 0 | | |
| Pattern of recurrence/metastasis | 0 | | |
| • • Liver/lung metastasis | 1 (16.7%) | | |
| 0 • Peritoneal metastasis | 4 (66.7%) | | |
| 0 • Lymph node metastasis | 1 (16.7%) ** | | |
| Follow-up (years) | 2.6 ± 2 | 4.4 ± 2.3 | |
| 3-year survival | 83.3% *** | 100% | |
| **Biomarker positivity** | | | |
| HB-EGF | 6/6 (100%) | 6/12 (50%) | 0.0339 |
| Integrin-α5 | 3/6 (50%) | 3/12 (25%) | 0.2900 |
| EGFR | 3/6 (50%) | 5/12 (42%) | 0.7400 |

TAHBSO: transabdominal hysterectomy and bilateral salpingo-oopherectomy, MMMT: Malignant Mixed Mullerian Tumor

*The case with omentectomy for operative staging had negative peritoneal washing cytology

**The previous pelvic lymph nodes dissection was negative for this patient. The new positive lymph node is outside pelvis

***This patient who died of disease had a MMMT with serous carcinoma component

**Table 2**

Histologic subtypes and H-score of biomarkers in each stage of Malignant Mixed Mullerian Tumor (MMMT)

| | HB-EGF | | | | EGFR | | | Integrin α5 | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | 0 | 1+ | 2+ | 3+ | 0 | 1+ | 2+ | 0 | 1+ | 2+ | 3+ |
| Serous (n=26) | | | | | | | | | | | |
| I w/o RM (n=1) | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 0 |
| I with RM (n=5) | 0 | 2 | 2 | 1 | 3 | 1 | 1 | 2 | 1 | 2 | 0 |
| II (n=1) | 0 | 0 | 1 | 0 | 0 | 1 | 0 | 0 | 1 | 0 | 0 |
| III (n=8) | 3 | 1 | 4 | 0 | 5 | 2 | 1 | 3 | 5 | 0 | 0 |
| IV (n=11) | 1 | 3 | 6 | 1 | 3 | 6 | 2 | 3 | 5 | 2 | 1 |
| subtotal | 4(15%) | 6(23%) | 14(54%) | 2(8%) | 12(47%) | 10(38%) | 4(15%) | 8(31%) | 13(50%) | 4(15%) | 1(4%) |
| Endometrioid(n=21) | | | | | | | | | | | |
| I w/o RM (n=6) | 3 | 1 | 2 | 0 | 1 | 5 | 0 | 4 | 1 | 1 | 0 |
| I with RM (n=1) | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 |
| II (n=3) | 2 | 0 | 1 | 0 | 1 | 1 | 1 | 2 | 1 | 0 | 0 |
| III (n=9) | 4 | 2 | 3 | 0 | 6 | 3 | 0 | 4 | 2 | 3 | 0 |
| IV (n=2) | 2 | 0 | 0 | 0 | 1 | 1 | 0 | 0 | 1 | 0 | 1 |
| subtotal | 11(52%) | 4(19%) | 6(29%) | 0(0%) | 9(43%) | 11(52%) | 1(5%) | 11(52%) | 5(24%) | 4(19%) | 1(5%) |
| UM (n=15) | | | | | | | | | | | |
| I w/o RM (n=5) | 3 | 2 | 0 | 0 | 3 | 0 | 2 | 5 | 0 | 0 | 0 |
| I with RM (n=0) | | | | | | | | | | | |
| II (n=1) | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 0 |
| III (n=5) | 4 | 0 | 0 | 1 | 5 | 0 | 0 | 4 | 0 | 1 | 0 |
| IV (n=4) | 2 | 1 | 1 | 0 | 2 | 2 | 0 | 3 | 0 | 1 | 0 |
| subtotal | 10(67%) | 3(20%) | 2(13%) | 0(0%) | 11(74%) | 2(13%) | 2(13%) | 12(80%) | 1(7%) | 2(13%) | 0(0%) |
| *P* * | 0.275 | | | | 0.206 | | | 0.927 | | | |

UM: unclassifiable/miscellaneous; HB-EGF: heparin binding-epidermal growth factor like growth factor; EGFR: Epidermal Growth Factor Receptor.

*The *P* value is the two-tailed probability of null hypothesis: no difference in H-score among positively stained cases for each biomarker.

## Table 3

Pearson's correlation coefficients for histologic subtypes and biomarker positivity in Malignant Mixed Mullerian Tumor (MMMT) across stages

| **Biomarkers (correlation, *p*)** | **HB-EGF** | **Integrin- α5** | **EGFR** |
|---|---|---|---|
| **Histology subtype (n=)** | | | |
| Serous carcinoma (n=26) | 0.919 (p=0.027) | 0.470 (p=0.424) | 0.342 (p=0.573) |
| Endometrioid carcinoma (n=21) | −0.837 (p=0.077) | −0.240 (p=0.697) | −0.284 (p=0.643) |
| Unclassifiable/miscellaneous (n=15) | −0.749 (p=0.145) | −0.645 (p=0.240) | −0.318 (p=0.602) |

HB-EGF: heparin binding-epidermal growth factor like growth factor EGFR: Epidermal Growth Factor Receptor

Correlation is demonstrated by correlation coefficient. *P*: two tailed p value;