Mucinous cystic neoplasm of the pancreas with neuroendocrine cells and malignant stroma

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ABSTRACT

تعد الأورام الكيسية الموسينية مع سدى ساركومي خبيث أورام عدوانية نادرة. فقد تم دراسة القليل من الحالات. وقد أجريت دراسة لورم كيسي موسيني فيه سرطان غدي لابد وسرطان غدي غزوي مع بؤرة ساركومية سدوية عند امرأة تبلغ من العمر 40 عاما, وتم ملاحظة انتقال واضح من منطقة السرطان الغدي الى بؤر الساركومي أظهرت الأجزاء السدوية تفاعلية مناعية Cam CK7 and 5.2 داعمة المنشأ الظهاري للمناطق الساركومية. المناطق المرتبطة بالظهارة الورقية الكيسية الموسينية الحميدة الخلوية كانت ظاهرة وكانت تفاعلية مناعية AE1/3, CK7, CK20, CAM 5.2, EMA, CEA, and MSA. أن تحديد الخلايا العصبية الصماوية الكمثرية الشكل المشتتة, والتي أثبتت بWفاعلية مناعية قوية للكروموغرانين و السينابتوفيسين كان واضحا في التبطين الورقي الكيسي الموسيني الخلوي MCN وليس في الأجزاء الظهارية الخبيثة. أن النقاش حول الاكتشافات يعطي افتراض بأن هذه الخلايا الورقية بالكاد تظهر من الخلايا الأولية القادرة على التمايز المتباعد.

Mucinous cystic neoplasms (MCN) with malignant sarcomatous stroma are rare aggressive tumors and there are few recorded cases. We report a case of MCN that had adenocarcinoma in situ and invasive adenocarcinoma with foci of sarcomatous stroma in a 40-year-old woman. Clear transition from adenocarcinoma areas into sarcomatoid foci was noted. The stromal component showed immunoreactivity for CK7 and Cam 5.2 supporting epithelial origin of the sarcomatoid areas. Associated areas of cytologically benign MCN epithelium were present and were immunoreactive for positive staining with pan-cytokeratin (AE1/AE3), cytokeratin 7 (CK 7), cytokeratin 20 (CK 20), pan-cytokeratin (Cam 5.2), epithelial membrane antigen (EMA), muscle specific actin (MSA), and carcino-embryonic antigen (CEA). Interestingly, definite scattered pear-shaped neuroendocrine cells, as evidenced by strong immunoreactivity for chromogranin and synaptophysin, were identified in the cytologically benign MCN lining but not in the malignant epithelial component. We found that these tumor cells probably arise from a single precursor cell capable of divergent differentiation.

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Conventional mucinous cystic neoplasms (MCN) are classified as tumors of the exocrine pancreas. They include the spectrum of benign mucinous cystadenomas, borderline mucinous tumors, and mucinous cystadenocarcinomas. They occur most often in young to middle-aged women and with much less frequency in middle-aged to older men. They predominantly occur in the body or tail of the pancreas.[1,2] The histological features of conventional MCN are comprised of a columnar epithelium lining cystic spaces with a characteristic sub-epithelial ovarian-type stroma. This characteristic histologic pattern differentiates MCN from other lesions of the pancreas.[1] There is a spectrum of differentiation from adenomas to borderline mucinous tumors (low-grade malignant) to cystadenocarcinomas. The adenomas have histologically benign appearing columnar epithelium lining multiloculated cysts. Borderline tumors exhibit papillary projections and/or crypt-like invaginations; cellular pseudo stratification with crowding of slightly enlarged nuclei, and occasional mitoses similar to their ovarian counterparts. Mucinous cystadenocarcinoms are characterized by invasion of malignant epithelium

into the underlying stroma. The stroma, which is characteristic of these neoplasms, is similar to that of the ovary where the cells are densely packed with oval to elongate nuclei and commonly scant cytoplasm. Although most of these cases are benign, in rare circumstances, an adenocarcinoma component can be present. The presence of sarcomatoid stroma in these neoplasms is even more rare and only has been reported in very few cases.[3-7] Due to variability and overlap in the non-epithelial components of these tumors, some of these lesions were referred to by a number of terms such as anaplastic carcinoma, pleomorphic carcinoma, undifferentiated carcinoma, and sarcomatoid carcinoma. The morphology of the non-mucinous component varies from monomorphic spindle cell lesions, such as the lesion in our case, to pleomorphic epithelial, rhabdoid and squamous patterns.[3-9] Despite the different morphological appearance of these stromal components, these malignant cells have been shown to be immunoreactive for epithelial cell markers. These findings raised the issue of an epithelial cell origin.[4-7] The diagnosis of a malignant spindle cell component in a mucinous cystic neoplasm such as the one described in this case report can be quite difficult. This is due in part to the resemblance of the malignant spindle cell component to that of the benign ovarian-type stroma, which is characteristically present in MCN. In addition, while carefully examining the atypical mucinous component of a pancreatic neoplasm for stromal invasion, an observer might overlook the malignant nature of the underlying stroma. Moreover, these sarcomatoid areas can be very focal and therefore may be easily missed unless extensive sampling is carried out. It is very critical to recognize these sarcomatous areas because of the highly aggressive nature of these types of tumors. We are reporting a case in a 40 year-old woman who had a malignant sarcomatous stroma associated with a mucinous cystadenocarcinoma where the stromal sarcoma cells were immunoreactive for 2 different types of cytokeratin. We are also reporting an interesting finding where we noticed the presence of scattered chromogranin and synaptophysin immunoreactive pear-shaped cells only in the cytologically benign cystic component.

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Case Report. A 40-year-old Caucasian woman presented with vague right-sided pelvic pain. Past medical history was significant for unilateral breast infiltrating ductal carcinoma 2 years prior that was treated with bilateral mastectomy following the patient's request. Computed tomography showed multiple small cysts in the right ovary, but also incidentally revealed an 8 cm complex cystic mass in the tail of the pancreas (**Figure 1**). Positron emission tomography scan showed minimal increased activity with a standard uptake value of 2.0. Distal pancreatectomy and splenectomy were performed. There was an 8.0 cm well-circumscribed cystic mass in the tail of the pancreas. The mass was multi-loculated and filled with cloudy and viscous mucous. Only rare excrescences and nodules were present along the cyst wall linings from inside. Microscopically, the thick-walled capsules of the cysts were lined by complex, mucinous columnar epithelium. Papillary formations with variable nuclear atypia and complexity were present. Some papillae were lined by simple, single-cell layers of columnar cells with basally oriented, minimally atypical nuclei, while other papillae were comprised of multi-cell layers in a cribriform pattern with atypia. One convincing area of stromal invasion by adenocarcinoma cells was identified which measured less than 2 millimeters in maximum diameter (**Figure 2A**). No invasion to the surrounding pancreas was identified, and the surgical resection margins were negative. No vascular or perineural invasion were present. The underlying neoplastic epithelium were multiple foci of highly cellular sarcomatous stroma, which had a high mitotic index and moderate degree of

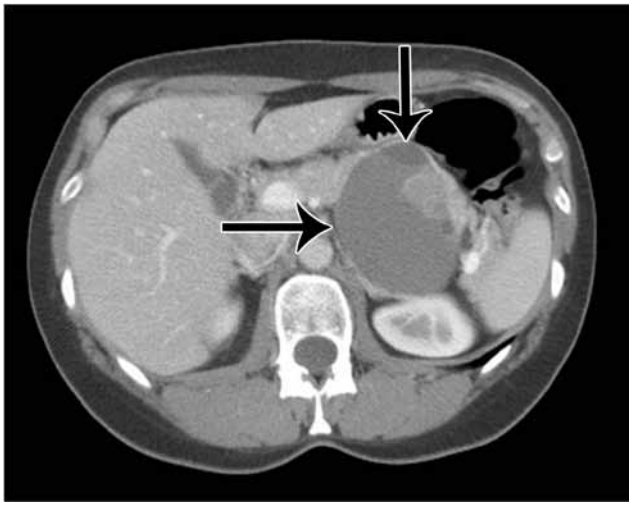

Figure 1 - Computerized tomography scan showing the complex pancreatic cystic neoplasm (arrows).

pleomorphism (**Figure 2B**). No glandular or squamoid areas of differentiation were present within the sarcomatous portions, nor were there any heterologous elements such as bone or cartilage. Interestingly, it was noted that in some areas there seemed to be a transition from the invasive carcinoma to the spindle cell sarcomatoid areas. Two peri-pancreatic lymph nodes were present which were negative for carcinoma as well as the spleen, which was unremarkable. Multiple well-controlled immunohistochemical stains for multiple antibodies were performed (BioGenex i6000, GMI Inc., Ramsey, MN) on formalin-fixed and paraffin-embedded tissue using the standard immunohistochemical staining techniques (**Table 1**). The mucinous cystic neoplastic epithelium showed positive staining with pan-cytokeratin (AE1/AE3), cytokeratin 7 (CK 7), cytokeratin 20 (CK 20), another pan-cytokeratin (Cam 5.2), epithelial membrane antigen (EMA), muscle specific actin (MSA) and carcino-embryonic antigen (CEA); but were negative for vimentin, S100 protein, neuron specific enolase (NSE), chromogranin and synaptophysin. There was obvious positivity for CK 7 and Cam 5.2 by the malignant stroma (**Figure 2C**). Interestingly, there were a few pear-shaped cells scattered in the mucinous cystic neoplastic epithelium, which were immunoreactive for chromogranin (**Figure 2D**) and synaptophysin. The more atypical columnar epithelium did not have these positive chromogranin and synaptophysin staining cells. Immunoreactivity for other neuroendocrine and hormonal markers such as insulin and glucagon were not performed. A summary of the immunohistochemical results of different components of this tumor is shown in **Table 2**.

The patient was discharged after 4 days with uneventful immediate post-surgical follow-up. She is alive and well and with no evidence of recurrence or metastasis as of the time of writing this report (48 months post surgery). Due to the previous history of breast carcinoma, a thorough family history was taken

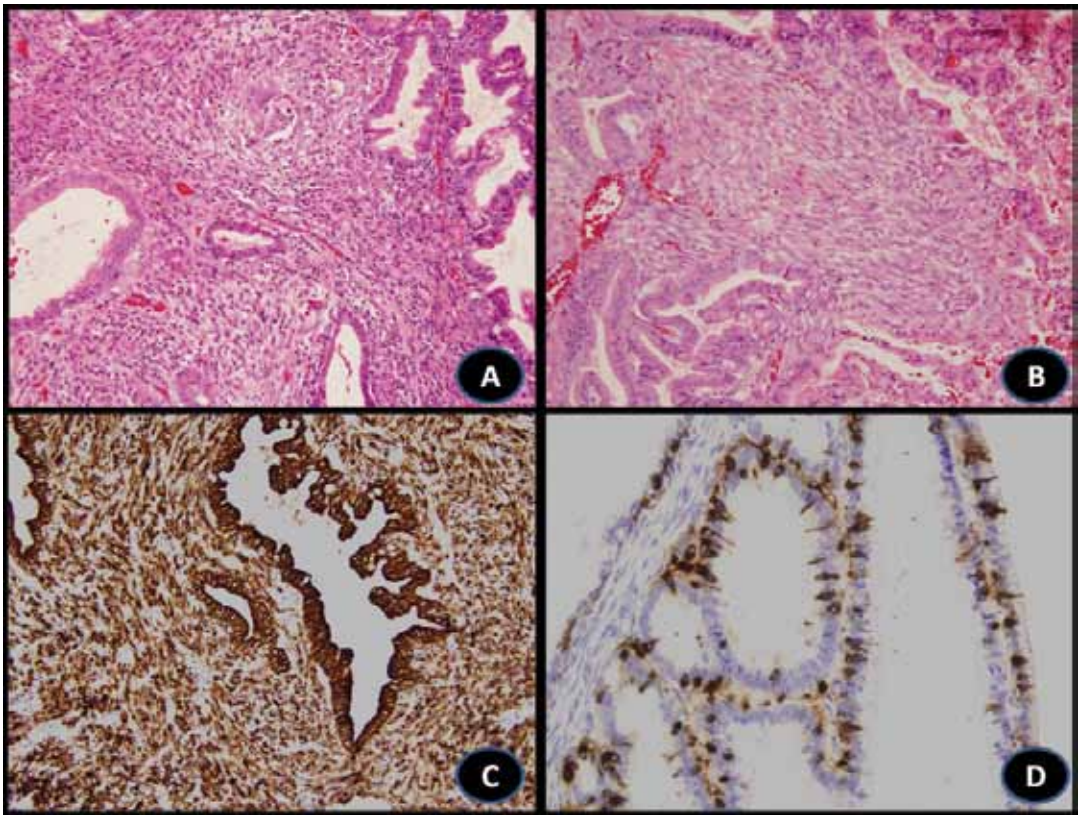

Figure 2 - Histological features **A**) Medium power view of the tumor showing invasive glandular areas and dense stroma in between representing the sarcomatoid areas (Hematoxylin & Eosin 200X). **B**) Another medium power view of the tumor showing frequent mitosis in the malignant sarcomatoid area of the tumor (Hematoxylin & Eosin 200X). **C**) Immunohistochemical staining for pan-cytokeratin (Cam 5.2) showing immunoreactivity of both the epithelial glandular and the sarcomatoid stromal components. **D**) Immunohistochemical staining for Chromogranin A showing positive scattered pear-shaped neuroendocrine cells in the cystic component of the neoplasm.

Table 1 - Multiple well-controlled immunohistochemical stains for multiple antibodies were performed (BioGenex i6000, GMI Inc., Ramsey, MN) on formalin-fixed and paraffin-embedded tissue using standard immunohistochemical staining techniques.

Antibody	BioGenex Code	Antigen retrieval	Enzyme pretreatment	Manufacturer	Clonality
Pan-cytokeratin (AE1/AE3)	AM071	None	Trypsin 20 min	BioGenex	Monoclonal
Pan-cytokeratin (CAM 5.2)	AM689	None	Prot K 6 min	Becton Dickinson	Monoclonal
Carcino-embryonic Antigen	AM687	None	Prot K 6 min	Dako	Polyclonal
Chromogranin A	AM126	None	Trypsin 20 min	BioGenex	Polyclonal
Cytokeratin 7	AM255	None	Prot K 6 min	BioGenex	Monoclonal
Cytokeratin 20	AM675	None	Prot K 6 min	BioGenex	Monoclonal
Epithelial membrane antigen	AM057	None	None	BioGenex	Monoclonal
Muscle specific antigen	AM681	None	None	Dako	Monoclonal
Neuron specific enolase	AM055	None	None	Dako	Monoclonal
S100 protein	AM058	None	None	BioGenex	Monoclonal
Synaptophysin	AM363	None	Prot K 6 min	Dako	Polyclonal
Vimentin	AM074	None	None	Dako	Monoclonal

Table 2 - Immunoreactivity for other neuroendocrine and hormonal markers such as insulin and glucagon were not performed. A summary of the immunohistochemical results of different components of this tumor.

Stain	Cytologically bland cystic neoplastic epithelium	Adenocarcinoma in situ	Invasive adenocarcinoma	Stromal sarcoma component
Pan-cytokeratin (AE1/AE3)	+	+	+	-
Cytokeratin 7	+	+	+	+
Cytokeratin 20	+	+	+	-
Pan-cytokeratin (Cam 5.2)	+	+	+	+
Epithelial membrane antigen	+	+	+	-
Carcino-embryonic antigen	+	+	+	-
Muscle specific antigen	+	+	+	+
Vimentin	-	-	-	+
S 100 protein	-	-	-	Weak focal +
Neuron specific enolase	-	-	-	-
Chromogranin	Few cells *	-	-	-
Synaptophysin	Few cells *	-	-	-

*Small scattered pear-shaped cells in the cytologically benign cyst lining

and analyzed and was negative; therefore, no further genetic testing was performed. Radiotherapy after the chemotherapy may not be necessary.

Discussion. Mucinous cystic adenocarcinomas of the pancreas with a malignant sarcomatous stroma are rare, but are considered aggressive tumors.[3-7] However, to the best of our knowledge and after careful review of the English language literature, the total number of such cases that have been reported was 7 cases (**Table 3**).[3-7] There are conflicting theories on the histogenesis that reflects on the different terminologies that are used to classify these lesions. The list includes anaplastic carcinoma, pleomorphic carcinoma, sarcomatoid carcinoma, and undifferentiated carcinoma. The morphologic appearance of the sarcomatous stroma that was observed and reported in the previous 7 cases that were published range from malignant spindle cells, pleomorphic, epithelioid, rhabdoid, osteoclastic, giant cell, and squamoid (**Table 3**). Our case had stroma consisting solely of malignant spindle cell component; however, with small areas that showed a transition to epithelioid morphology. Similar to other reports, the sarcomatous stroma can show immunoreactivity to different epithelial cell markers.[4-7] The sarcomatous stroma in our patient stained positive with CK7 and Cam 5.2. The staining with these epithelial markers supports the contention that these areas are essentially sarcomatoid (spindle) carcinomas and most likely arose from the same cell of origin. The combination of the CK7 and Cam 5.2 stromal positivity along with the positive stromal staining with the non-epithelial markers Muscle specific actin (MSA), Vimentin, and S100 (weakly positive) raises the possibility that the malignant spindle cells have "dual-differentiation".[10] Moreover, the epithelial cell component was immunoreactive for MSA further augmenting the dual differentiation theory.

A unique and unexpected finding in our case was the chromogranin and synaptophysin positive pear-shaped cells that were seen scattered among the cytologically

Table 3 - Previously reported cases of sarcomatous stroma arising in mucinous cystic neoplasm and their features.

Authors	Years	Number of cases	Age/gender	Morphology of sarcomatoid component	Immunohistochemistry of the spindle cell sarcomatous component	Follow up
Wenig et al[3,*]	1996	3	48/F 66/F 67/M	Spindle cell	Positive for vimentin and MSA; but all negative for epithelial markers	Alive after one year Died after 9 months Died after 15 months
Lane & Sangueza[4]	1996	1	25/F	Anaplastic morphology	Positive for vimentin and low molecular weight keratins	Alive 6 months after diagnosis
Nishihara et al[5]	1997	1	52/F	Anaplastic with rhabdoid features	Positive for vimentin and EMA	Died 19 months after diagnosis
Bloomston et al[6]	2006	1	67/F	Spindle cell morphology in addition to poorly differentiated and squamous areas	Positive for vimentin	Died 4 months after diagnosis
Pan & Wang[7]	2007	1	70/F	Anaplastic with predominant spindle cell component	Positive for vimentin and MUC-1 Focally positive for CK7 and CK 20	Alive 4 months after diagnosis
Asberry et al (present study)	2012	1	40/F	Spindle cell morphology	Positive for CK7, Cam 5.2, vimentin, MSA and weak focal staining for S100	Alive 48 months after diagnosis

*3 cases were re-studied by the same group (van den Berg et al[10]) where molecular analysis showed evidence of similar genetic changes between the epithelial and the sarcomatous components of these tumors. MSA - muscle specific actin, EMA - epithelial membrane antigen, MUC-1 - membrane-associated mucins 1, CK7 - cytokeratin 7, CK20 - cytokeratin 20

benign neoplastic epithelial cell lining of this tumor and not seen in the malignant (adenocarcinoma in situ or invasive adenocarcinoma) part. If we consider these markers' positivity an indication of focal neuroendocrine differentiation, we can only speculate that the cell of origin may harbor the ability of divergent cell-type differentiation (cells are multipotent in nature). This theory has also been supported by molecular evidence. Van den Berg et al[10] analyzed 3 MCN with sarcomatous stroma for 6 microsatellite markers commonly deleted in pancreatic ductal adenocarcinomas. In 2 of the 3 cases, the genetic alterations were virtually identical between the epithelial and sarcomatous components. The sarcomatous and epithelial components of the third case were also identical in 5 of the 6 chromosomal loci. Those findings lend further credence to the theory that a monoclonal origin with subsequent divergence of the malignant epithelial and sarcomatous portions maybe a valid scenario. It is worth mentioning, as noted in our case, that the invasive carcinoma as well as the presence of sarcomatous areas, can be very focal. Therefore, extensive sampling is extremely critical in these tumors and cannot be overemphasized. These lesions are aggressive neoplasms with worse prognoses than the mucinous cystadenocarcinoma without a malignant stroma, and fortunately are very rare. In contrast to the cases published in the literature, our patient is still alive and well 48 months after surgery with no evidence of recurrence. We have a reason to believe that our patient is cured for the following reasons: she had a very small focus of malignant component, there was no extra-pancreatic extension by the tumor, all the surgical margins of resection were negative, and the 2 peri-pancreatic lymph nodes that were found were both negative.

In conclusion, the findings in our report and also those of others suggest that the malignant stroma originates from an epithelial precursor and hence better be classified as "sarcomatoid carcinoma". The presence of neuroendocrine cells in conjunction with the benign mucinous cystic neoplastic lining strongly suggests a multipotent stem cell origin of these tumors. Extensive sampling in such tumors is highly recommended since malignant foci can be very focal.

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