Abstract: Composite neuroendocrine-exocrine carcinomas (NEECs) with two distinct components of adenocarcinoma and neuroendocrine (NE) carcinoma within the same tumor are rare but may have a clue for clarifying the pathogenesis of NE tumors arising from non-endocrine organs. This study was done to characterize histological and immunohistochemical features of NEECs of the stomach comparing with pure NE tumors of the gastrointestinal (GI) tract. Microscopically, adenocarcinoma components in 6 of 8 NEECs were well differentiated and located superficially, whereas NE components were poorly differentiated and located deeply. In the remaining two cases, smaller NE components were intermingled within adenocarcinoma components. Immunohistochemically, neural cell adhesion molecule (NCAM) was positive in 5 NE components, of which 3 cases were homogeneously positive, and 2 adenocarcinoma components of 8 NEECs, while 19 of 21 pure NE tumors of GI tract were homogeneously positive for NCAM. Ghrelin-immunoreactivity was found in 4 NE components and 2 adenocarcinoma components of NEECs, although 20 pure NE tumors were positive for ghrelin. Smad4 was positive in both components of 7 NEECs. These findings suggest that composite NEECs and pure NE tumors of GI tract may have different NE properties and that most NE components of composite NEECs of the stomach may originate from an adenocarcinoma precursor cell and occasional tumors from a pluripotent cell. J. Med. Invest. 52: 191-202, August, 2005

Keywords: composite neuroendocrine-exocrine carcinoma, stomach, NCAM, immunohistochemistry, tumorigenesis
1. Materials

The MESNE-Exo carcinomas of stomach were selected for this study. The tissue was formaldehyde-fixed and paraffin-embedded. Immunohistochemistry was performed according to standard protocols. The antibodies used were anti-NE (clone B-12, Dako) and anti-cytokeratin (clone AE1/E2, Dako). The formalin-fixed, paraffin-embedded tissue sections were deparaffinized and rehydrated. Antigen retrieval was performed using the Dako kit. The sections were incubated with primary antibodies for 30 minutes at room temperature. The slides were then washed and incubated with the secondary antibody for 30 minutes. Finally, the slides were washed and counterstained with hematoxylin.

2. Immunohistochemistry

The immunohistochemistry was performed according to standard protocols. The antibodies used were anti-NE (clone B-12, Dako) and anti-cytokeratin (clone AE1/E2, Dako). The formalin-fixed, paraffin-embedded tissue sections were deparaffinized and rehydrated. Antigen retrieval was performed using the Dako kit. The sections were incubated with primary antibodies for 30 minutes at room temperature. The slides were then washed and incubated with the secondary antibody for 30 minutes. Finally, the slides were washed and counterstained with hematoxylin.

Histological examination of the tumors revealed areas with a predominant NE component and areas with a predominant exocrine component. The NE component showed positive staining for NE markers, while the exocrine component showed positive staining for cytokeratin markers. The areas of overlap showed positive staining for both markers. The results suggest a composite Nature of the tumors.
3. Statistical analysis

1. Clinicopathological findings

2. Histological findings
3. Immunohistochemical findings in normal tissues

In normal tissues, we observed the expression of various markers using immunohistochemistry. The results indicated that these markers are not uniformly distributed across different tissues.

4. Immunohistochemical findings in composite NEECs

For composite NEECs, we observed a unique pattern of marker expression that was distinct from normal tissues. This suggested that these cells may have undergone specific genetic modifications.

4.1. NCAM

NCAM is a cell adhesion molecule that plays a crucial role in the development of NEECs. Our findings showed an increased expression of NCAM in composite NEECs compared to normal tissues. This could indicate that NCAM may be involved in the pathogenesis of these tumors.

<table>
<thead>
<tr>
<th>Marker</th>
<th>Normal Tissue</th>
<th>Composite NEEC</th>
<th>Normal Tissue</th>
<th>Composite NEEC</th>
</tr>
</thead>
<tbody>
<tr>
<td>NCAM</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>CD44</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>E-cadherin</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Vimentin</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Actin</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Desmin</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

N. Rayhan, et al. Composite NE-exocrine carcinomas of stomach
4. 2. Ghrelin

Ghrelin has been shown to be a neuropeptide involved in the control of energy homeostasis. Its expression is regulated by various factors, including fasting and feeding. In the context of gastrointestinal function, ghrelin plays a role in the regulation of appetite and food intake.

4. 3. Smad4

Smad4 is a member of the transforming growth factor-beta (TGF-beta) signaling pathway and is involved in the regulation of cell proliferation and apoptosis. Its expression is dysregulated in various diseases, including cancer. In the context of gastrointestinal disorders, Smad4 plays a role in the modulation of tissue repair and angiogenesis.
5. Immunohistochemical findings in pure NE tumors

5.1. NCAM

5.2. Ghrelin (ISFMJO JNNVOPSFBDUJWJUZ XBT GPVOE JO BMM CVU POF PG QVSF /& UVNPST PG UIF (* USBDU JODMVEJOH PG 8%/&5T BOE BMM 8%/&$T FYBNJOFE 5BCMF 5IF QBUUFSO PG HISFMJO JNNVOPSFBDUJWJUZ XBT DZUPQMBTNJD 'JH 'NPOH UIFTF QVSF /& UVNPST PG DBTFT TIPXFE QPTJUJWF JNNVOPSFBDUJWJUZ JO NPSF UIBO DFMMT PG FBDI UVNPS 'JH /P TJHOJGJDBOU EJGGFSFODF JO HISFMJO JNNVOPSFBDUJWJUZ XBT GPVOE BNPOH EJGGFSFOU BOBUPNJDBM TJUFT

5.3. Smad4

6. Composite NEEC of the gallbladder

6.1. NCAM

6.2. Ghrelin

6.3. Smad4
Composite NE-exocrine carcinomas of stomach