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High-grade Transformation/Dedifferentiation in Salivary Gland Carcinomas: Occurrence Across Subtypes and Clinical Significance

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Abstract: High-grade transformation (HGT) or dedifferentiation has been described in a variety of salivary gland carcinomas, including acinic cell carcinoma, secretory carcinoma, adenoid cystic carcinoma, epithelial-myoepithelial carcinoma, polymorphous adenocarcinoma, low-grade mucoepidermoid carcinoma, and hyalinizing clear cell carcinoma. High-grade (HG) transformed tumors are composed of a conventional low-grade component characterized by specific microscopic and immunohistochemical features for the given entity, intermingled with or juxtaposed to areas of HG morphology. This is usually either poorly differentiated adenocarcinoma, carcinoma not otherwise specified, or undifferentiated carcinoma, in which the original line of differentiation is lost. The HG component is composed of solid nests of anaplastic cells with large vesicular pleomorphic nuclei, prominent nucleoli, and abundant cytoplasm. Frequent mitoses and extensive necrosis may be present. The Ki-67 labeling index is consistently higher in the HG component. The molecular genetic mechanisms responsible for HGT of salivary gland carcinomas are largely unknown, though p53 inactivation and human epidermal growth factor receptor 2 overexpression and/or gene amplification have been demonstrated in the HG component in a few examples, the frequency varies for each histologic type. Salivary gland carcinomas with HGT are more aggressive than conventional carcinomas, with a higher local recurrence rate and a poorer prognosis. They have a high propensity for cervical lymph node metastasis suggesting a need for a wider resection and neck dissection. HGT of salivary gland carcinoma can occur either at initial presentation or less commonly at the time of recurrence, sometimes following postoperative radiotherapy. The potential for HGT in almost any type of salivary gland carcinoma warrants a thorough sampling of all salivary gland malignancies to prevent oversight of a HG component.

Key Words: salivary gland carcinoma, high-grade transformation, dedifferentiation, p53, *HER2*/neu

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The concept of high-grade transformation (HGT) in neoplasms, previously also called "dedifferentiation," was introduced in 1971 by Dahlin and Beabout,[1] who described a distinct clinicopathologic entity in which a low-grade (LG) chondrosarcoma was associated with, but sharply delineated from, a histologically dissimilar high-grade (HG) sarcoma. Subsequently, "dedifferentiation" has become a well-known phenomenon in bone and soft tissue pathology.[2] After the first report of a "dedifferentiated" salivary gland acinic cell carcinoma (AciCC) in 1988,[3] several other investigators reported this phenomenon not only in AciCC[4–7] but also in other salivary gland malignancies, such as secretory carcinoma (SC), also known as mammary analogue SC,[8,9] adenoid cystic carcinoma (AdCC),[10–12] epithelial-myoepithelial carcinoma (EMC),[13,14] polymorphous low-grade adenocarcinoma (PLGA) [recently called polymorphous adenocarcinoma (PAC)],[15] LG mucoepidermoid carcinoma (MEC),[16] and hyalinizing clear cell carcinoma (CCC).[17] The concept of HGT in salivary neoplasms has been widely accepted during the last decade, and the number of reported cases is rapidly increasing. Histologically, the HG component of the tumor is generally composed of solid nests, sometimes occurring in the cribriform pattern, of anaplastic cells with large vesicular pleomorphic nuclei, prominent nucleoli, and abundant cytoplasm. There are frequent mitoses and a central comedo-type of necrosis. Ki-67 (MIB-1) labeling index is always higher in the HG component.[6–8,18]

HGT is defined as the abrupt transformation of LG "well-differentiated" carcinoma into HG morphology that lacks the original distinct histologic and immunohistochemical features.[18] The conventional LG and HG areas are usually clearly demarcated, although a transitional zone may be present in some cases. Although HGT and dedifferentiation have been used synonymously in salivary gland pathology, it has become more recognized that while some tumors still retain their diagnostic morphologic and/or immunophenotypic diagnostic features in the HG component and hence are merely HG transformed, others display a nondescript undifferentiated morphology and immunophenotype throughout the HG component and thus fulfill the definition of "dedifferentiation." In salivary gland pathology, unlike in bone and soft tissue sarcomas, some features of the original line of differentiation are frequently retained in the HG component, therefore, "high-grade transformation" seems a more appropriate designation than "dedifferentiation."

The molecular genetic mechanisms responsible for HGT of salivary gland carcinomas are still largely unknown. The development and progression of malignant tumors is regulated by genetic and/or epigenetic alterations with activation of oncogenes and inactivation of tumor suppressor genes. Although the data is limited, the involvement of one or several apparently randomly altered genes has been documented in the HGT process of salivary gland tumors. These alterations include mutation and/or amplification of oncogenes, loss of tumor suppressors, or combinations of both.

HGT of a neoplasm can also be associated with epigenetic events, such as DNA hypomethylation or hypermethylation leading to overexpression or inactivation of specific genes. Such events could result in chromatin decondensation and instability, an increased occurrence of chromosomal rearrangements, and silencing of genes critical for the control of cell growth.[19] However, there is extremely little information available about epigenetics in salivary gland tumors, and its possible role in triggering/inhibiting the activity of established genetic modifications or effects on tumor suppressor genes. Interestingly, one study reported that *CLIC3* was differentially methylated between salivary MEC and normal salivary gland.[20] This observation together with the comprehensive evidence of the role of epigenetics in the development and progression of other cancers further emphasize the need for additional studies of the epigenetic landscape of salivary gland tumors including those showing HGT. Taken together, the exact molecular (genetic/epigenetic) mechanisms underpinning HGT in salivary gland carcinomas are still largely unknown, partly because of the alleged rarity of this phenomenon.

HGT in salivary carcinomas is almost always associated with aggressive clinical behavior and a poor prognosis, irrespective of the histologic type of the original tumor. There is a high propensity of salivary carcinomas with HGT to metastasize to cervical lymph nodes, and this suggests a need to include neck dissection in the therapy. HGT of salivary gland carcinomas can occur either at initial presentation or less commonly at the time of recurrence, sometimes following postoperative radiotherapy. In practice, the potential for HGT in almost any type of salivary gland carcinoma warrants a thorough sampling of all salivary gland tumors to avoid overlooking an HG component. In contrast, the possibility of HGT of a specific entity should be considered in undifferentiated or unclassifiable HG carcinomas on biopsies with limited tumor tissue. On resections, thorough sampling has often proved highly valuable in recognizing a minor differentiated tumor component that enables a precise classification. HGT/dedifferentiation in salivary gland carcinomas has been the subject of recent excellent reviews by Nagao,[18] Costa et al,[21] and Petersson.[22]

ACINIC CELL CARCINOMA

AciCC is an uncommon carcinoma of the parotid gland and much less frequently of other major and minor salivary glands, accounting for ~6% to 7% of all salivary gland neoplasms.[7,23] Serous acinar cell differentiation, which is characterized by cytoplasmic zymogen (secretory) granules is the principal diagnostic feature. AciCC with HGT is very rare and is defined as being composed of a conventional LG and a morphologically HG component; the latter may consist of HG adenocarcinoma, poorly differentiated carcinoma, small cell carcinoma, or undifferentiated carcinoma. HGT in AciCC can progress from any conventional AciCC pattern to an HG carcinoma typically lacking acinic differentiation.

Stanley et al[3] published the first series of 6 cases of AciCC-HGT originally reported as "dedifferentiation" in 1988, and recognized their aggressive clinical behavior. Since then, AciCC-HGT has been reported increasingly.[4–7,23–26] The parotid gland was affected in all reported AciCC-HGT.[7] The mean age of 61 to 66 years observed in patients with AciCC-HGT is ~20 years higher than that of patients with conventional AciCC.[6,7,25–27] AciCC-HGT presents with a slight female predominance, similar to that of conventional AciCC.

In most cases of AciCC-HGT, the HG component is a poorly differentiated adenocarcinoma (Fig. 1A). The HG component appears as a distinct population of anaplastic cells with abundant cytoplasm and large polymorphic nuclei (Fig. 1B). Irregular-shaped tumor islands which in places have geographic and/or comedo-type necrosis infiltrate a desmoplastic stroma devoid of lymphoid cells (Fig. 1C). Tumor cells of the HG component fail to reveal any acinar differentiation, while the LG component usually comprises solid and microcystic growth patterns. Periodic acid-Schiff stain reveals cytoplasmic granules resistant to diastase digestion consistent with the zymogen granules of LG AciCC (Fig. 1D). Intravascular and perineural invasion by the HG carcinoma cells is often observed. Interestingly, the metastatic foci may be composed of both LG and HG components.[6]

Immunohistochemically, the HG component in AciCC-HGT cases demonstrates an increased expression of the regulatory markers cyclin D1 and Ki-67, as compared with conventional LG AciCC.[6,7] Similarly, aberrant p53 immunoreactivity patterns indicating mutations are frequent in transformed cases as compared with LG AciCC.[6,7] While the luminal membranous expression of DOG1 is typically seen in LG AciCC and helps to highlight minor easily overlooked LG foci,[28] it is usually lost in HG areas.[6] SOX10 expression is typically seen in AciCC and mostly is retained in AciCC-HGT as well. The HG component, in contrast to conventional AciCCs, has high mitotic activity (Fig. 1E). Recently, nuclear reactivity with the NR4A3 protein (NOR-1) as a novel ubiquitous marker for AciCC was shown to be retained in the HG component indicating a valuable adjunct

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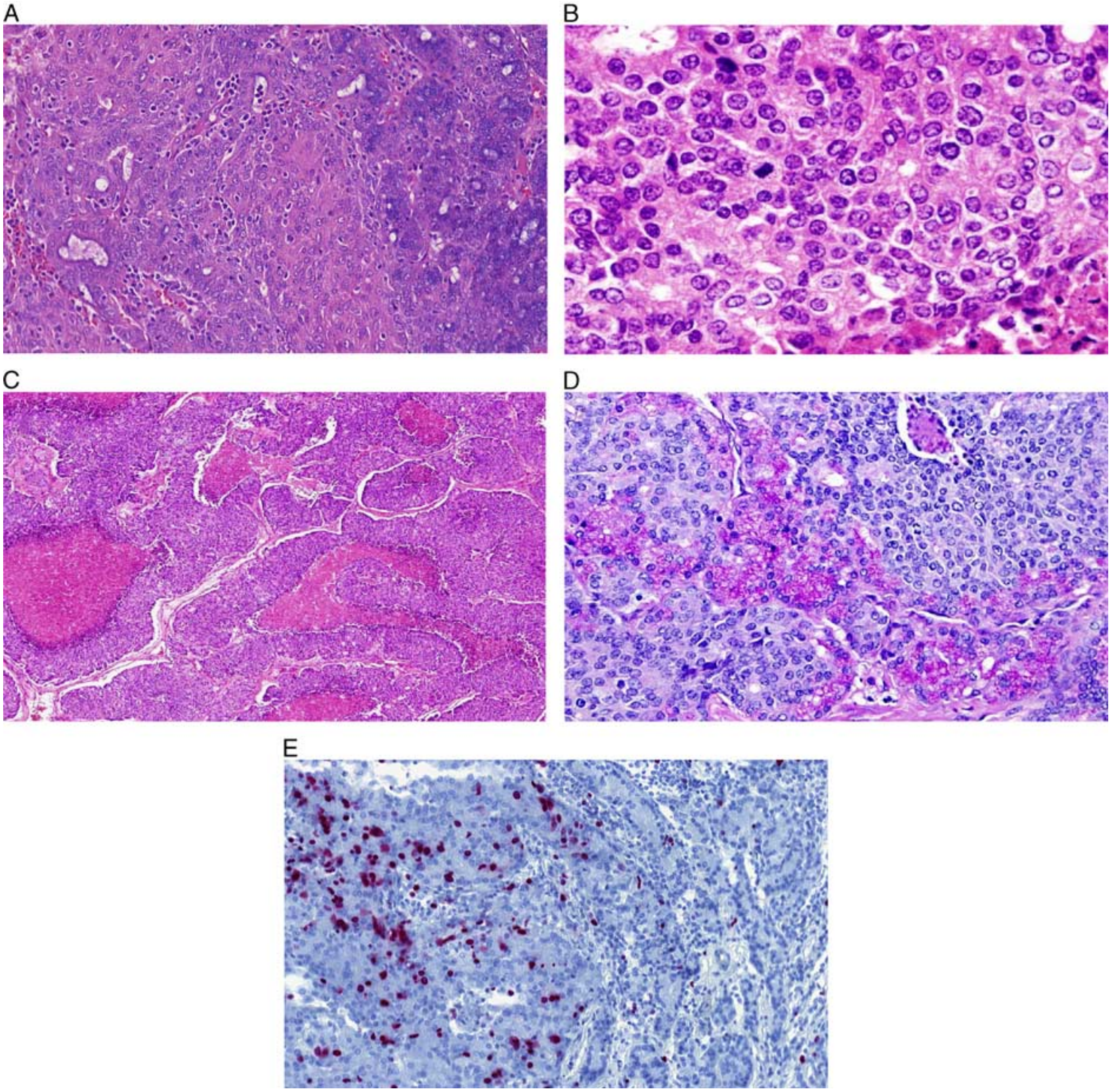

FIGURE 1. High-grade transformation of acinic cell carcinoma (AciCC). Low-power view of 2 components with conventional AciCC (right portion) and high-grade carcinoma with solid pattern of growth (left portion) (A). The high-grade component appears as a distinct population of anaplastic cells with abundant cytoplasm and large polymorphic nuclei (B). Irregular-shaped tumor islands in places with geographic necrosis infiltrates a desmoplastic stroma devoid of lymphoid cells (C). Periodic acid-Schiff stain reveals cytoplasmic granules resistant to diastase digestion consistent with the zymogen granules of low-grade AciCC (D). The high-grade component displays a high Ki-67 labeling index (left portion), in contrast to a low index in conventional AciCC (right portion) (E).

to recognize AciCC-HGT on biopsies and in resection samples without detectable LG component, although only limited cases have been studied.[29]

The behavior of AciCC-HGT is significantly worse than in patients without HGT, leading to an accelerated clinical course often resulting in dissemination of cancer and death of the patient. Such an aggressive clinical course was observed even in cases where HG areas represented no $>5\%$ of the primary lesion.[6] Therefore, we conclude that a tumor with HG foci of any size should qualify as an AciCC-HGT. In view of the aggressive nature of the AciCC-HGT, radical surgery and adjuvant radiotherapy are recommended. In particular and unlike conventional AciCC, AciCC-HGT seems to have a high propensity for cervical lymph node metastasis suggesting a need for neck dissection in the management of patients. Another implication for the surgical pathologist is that resected specimens of all AciCCs should be thoroughly sampled to avoid missing an HG component, particularly in cases of recurrence, as failure to recognize areas of HGT in an AciCC may result in mismanagement of the patient.[6]

Secretory Carcinoma (Mammary Analogue)

SC is an LG salivary gland malignancy with a characteristic *ETV6-NTRK3* fusion, originally designated mammary analogue SC.[30] HGT has been described to date in 10 patients with SCs including the present case, see below.[8,31–35] The tumors were localized in the parotid gland (n = 3), the submandibular gland (n = 1), the palate (n = 2), the buccal mucosa (n = 1), the maxillary sinus (n = 1), unspecified (n = 1), and the lower lip (n = 1, present case of 76 y old man, see below).

Histologically, conventional SC typically exhibits papillary-cystic, microcystic, tubular, or solid architecture, and is composed of uniform cells with bland vesicular nuclei and eosinophilic to vacuolated cytoplasm.[30] SC-HGT reveals an HG tumor component, characterized by trabecular or solid growth pattern, nuclear anaplasia/pleomorphism, and prominent geographic necrosis which is usually identified juxtaposed to the conventional SC component (Figs. 2A–D). While immunoexpression of pankeratin antibodies (AE1/AE3), CK7, CK8, CK18, CK19, vimentin, S-100 protein, and mammaglobin is mostly retained in the HG component,[8] other markers like MUC4 are frequently lost.[36]

In a recent study of 49 SCs analyzed by next-generation sequencing, one recurrent SC-HGT revealed dual *ETV6-NTRK3* and *MYB-SMR3B* fusion transcripts.[35] The patient was a 61-year-old man who presented with a recurrent tumor of the right submandibular gland measuring 1.9×1.5×1.0 cm. The affected salivary gland was resected and the patient underwent radiotherapy. Four years later, the patient developed a tumor recurrence in adjacent tissues in the right side of the neck. Lymph node dissection was performed and a single metastatic lymph node was found among 41 investigated neck lymph nodes. Fourteen months later, a new scar nodule was noted and confirmed to be a recurrent carcinoma.[35]

An additional case of SC-HGT (not published so far) was a 76-year-old man with a carcinoma arising in a minor salivary gland of the lower lip. It displayed a locally invasive growth pattern, with lymphatic and perineural invasion. The tumor was incompletely excised with positive margins, and its recurrence after 18 months was removed surgically followed by radiotherapy. Despite maximum treatment, the patient developed lung metastasis and he died 20 months after primary surgery.

Although only a small number of cases have been described in the literature, it appears that SC-HGT is associated

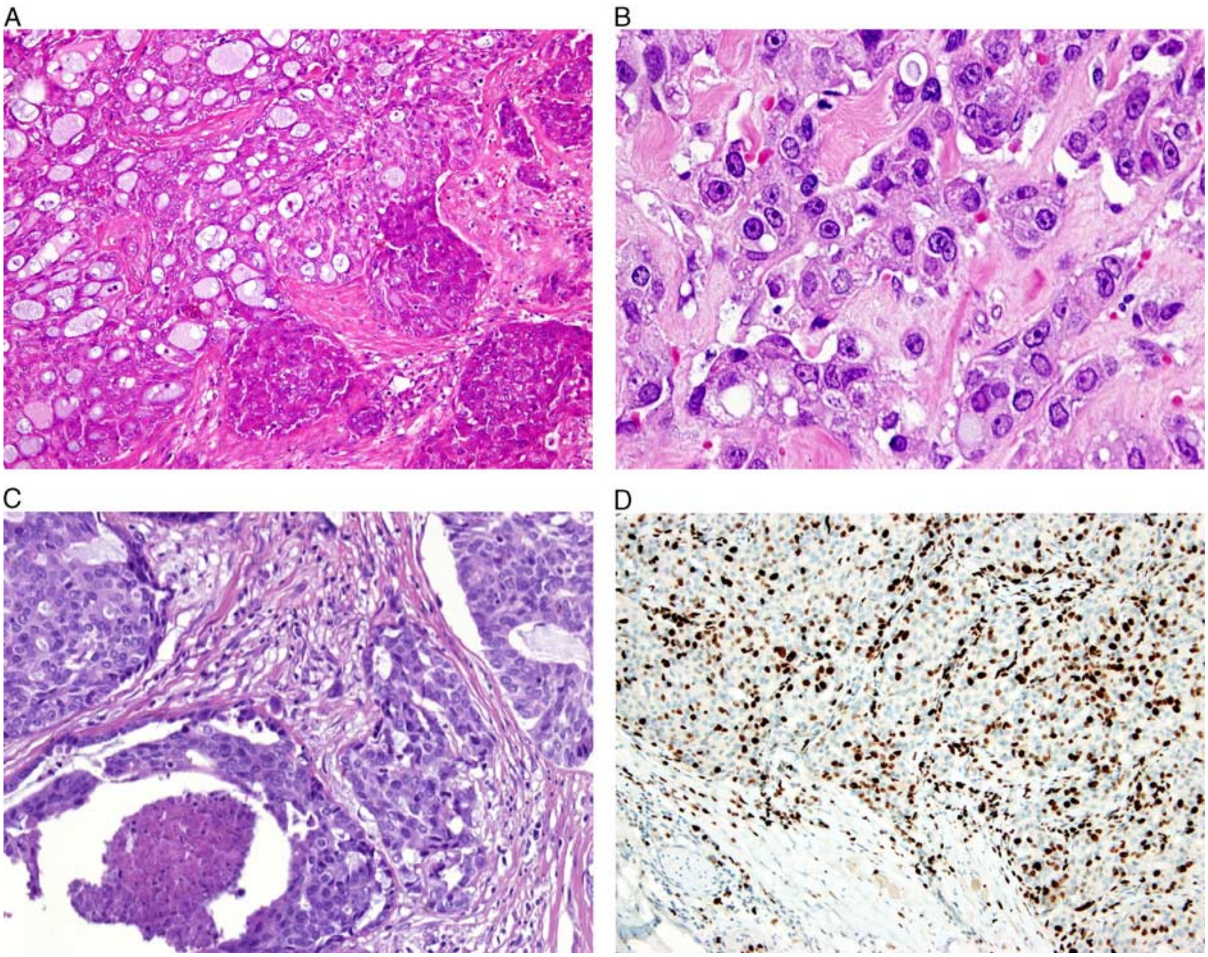

FIGURE 2. High-grade transformation of secretory carcinoma. Low-power view shows 2 distinct sharply delineated carcinomatous components. Conventional low-grade secretory carcinoma reveals a characteristic microcystic and vacuolated pattern (left portion), whereas the high-grade component shows a solid growth without secretory activity (right portion) (A). The high-grade component appears as a distinct population of anaplastic cells with abundant cytoplasm and large polymorphic nuclei and prominent nucleoli (B). Irregular-shaped tumor islands with comedo-like necrosis are present in high-grade component (C). High proliferative activity is present with MIB-1 labeling index > 60% (D).

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with an adverse clinical outcome. Five patients with SC-HGT died of disseminated distant metastasis, 3 from a parotid primary,[8] and one each from a tumor of the lip (current case) and the buccal mucosa.[33] Local recurrence developed within 4 months to 6 years after the initial diagnosis, and 4 patients presented with lymph node metastasis at the time of the initial resection, or with a recurrent tumor.[8,33–35]

A specific chromosomal translocation t(12;15)(p13; q25) resulting in *ETV6-NTRK3* gene rearrangement was initially considered as a distinct molecular signature of SC.[30] However, more recent studies have raised the possibility of alternative *ETV6* partners, first published as SCs with *ETV6-X* gene fusions.[37,38] Subsequently, one such alternative rearrangement in a subset of SC was shown to be an *ETV6-RET* gene fusion.[39] Importantly, in the first series of Skálová et al,[8] the *ETV6-NTRK3* gene fusion was demonstrated in both the LG and HGT component.

In summary, while the initial description of SC included only the *ETV6-NTRK3* gene fusions (still the most frequently identified), since then, other alternative chimeric fusions have been reported including *ETV6-RET*, *ETV6-MET*, dual *ETV6-NTRK3*, and *ETV6-MAML3*.[39–41] Although most SCs are slow-growing, LG malignancies that can be effectively managed by surgery alone, a small subset of SCs may transform into aggressive HG carcinomas.[8] SCs with the classic *ETV6-NTRK3* gene fusion are well-known to respond to the tyrosine kinase (TRK) inhibitors vitrakvi (larotrectinib) and rozlytrek (entrectinib) or to the second-generation TRK inhibitors selitrectinib (LOXO-195)[42] and repotrectinib.[43] However, a small subset of SCs reveals alternative gene fusions different from *ETV6-NTRK3*, such as *ETV6-RET*[39] and *VIM-RET*.[35] The *RET* fusion-positive tumors would obviously be unreactive to all first and second-generation TRK-specific inhibitors. However, specific *RET* inhibitors, such as selpercatinib (LOXO-292) and pralsetinib (Blu-667) are under development, and they have been shown to be effective in a number of *RET*–fusion-positive tumors, and are expected to be effective in *RET*–fusion-positive SCs as well.[44] Identification of these novel rearrangements expands the molecular diversity of SCs and opens a possibility for integrating targeted *RET* inhibitors in their therapeutic strategy. This emphasizes that in SC-HGT (and indeed, in any SC displaying aggressive behavior), full characterization of the gene rearrangement should be performed, if at all possible.

Adenoid Cystic Carcinoma

AdCCs are common malignancies arising in major and minor salivary glands, including the seromucinous glands of the upper respiratory tract and the lacrimal glands. AdCCs are characterized clinically by slow but persistent and relentless growth, a relatively high rate of local recurrence, and late onset of distant metastasis. Histologically, AdCCs may exhibit a mixture of 3 distinctive growth patterns: cribriform, tubular, and solid. The predominant growth pattern is predictive to some extent of the clinical outcome. AdCCs with both tubular and cribriform patterns generally have a better prognosis than those with solid pattern. The presence and extent of the solid pattern (currently with a cutoff of 30%) portend an accelerated and worse clinical course.[45,46] Since the original description of 3 cases of HGT/dedifferentiation in AdCCs (both de novo and in recurrences) by Cheuk et al,[10] > 50 cases have now been published.[11,12,47–55]

The histologic hallmarks of HGT in AdCC include necrosis, high mitotic rate, and loss of the biphasic ductal-myoepithelial differentiation.[10] It is mainly the cytomorphologic features that allow for a distinction between AdCC-HGT and the solid pattern of AdCC. Although a higher degree of nuclear atypia, a higher number of mitotic figures, and focal necrosis can be encountered in solid AdCCs, the neoplastic cells still have scanty cytoplasm and rather uniform nuclear features. In contrast, the cells of AdCC-HGT display a noticeable variation of nuclear size and shape, vesicular chromatin, increased nucleolar size, and more abundant cytoplasm. Moreover, solid AdCC often intermingles with classic tubular and cribriform patterns instead of occupying a "compartment" separate from the non-HG component (Figs. 3A–C).

Immunohistochemically, the HG component has shown a loss of expression of myoepithelial markers with the notable exception of one published case of AdCC-HGT with an HG anaplastic myoepithelial carcinoma (MC) of the lacrimal gland.[53] The Ki-67 index is significantly increased in the HG component compared with conventional tubular and cribriform AdCC (Fig. 3D).

The pathogenesis of HGT in AdCC is not well understood, although p53 alterations may play a significant role in the evolution of this phenomenon.[12] The literature on the status of *TP53* in AdCC-HGT is limited, but Chau et al[48] have demonstrated a *TP53* mutation selectively in the HG component. Furthermore, Nagao et al[11] demonstrated loss of heterozygosity at *TP53* microsatellite loci in 2 cases and a *TP53* point mutation in 1 case, all selectively in the HG component. In contrast to AciCC-HGT, AdCC-HGT shows immunohistochemical overexpression of p53 in a significant proportion (~50%) of cases. Interestingly, diffuse/strong immunoexpression of the human epidermal growth factor receptor 2 (HER2) protein has been reported in 3 cases of AdCC-HGT.[11,49] In addition, loss of Rb protein expression has been documented in 1 case, which was negative for overexpression of p53 and HER2.[11] C-kit (CD117) is frequently positive in both neoplastic components in AdCC-HGT.[12] An increased expression of *GLUT1* has been reported in the HG component in a few cases of AdCC-HGT.[52,56]

The most common molecular genetic alteration that characterizes AdCC is a recurrent translocation t(6;9)(q22-23;p23-24) that results in fusions encoding chimeric transcripts predominantly consisting of *MYB* exon 14 linked to the last coding exon(s) of *NFIB*.[57] Using high-resolution microarray comparative genomic hybridization, Costa et al[58] studied 6 cases of AdCC-HGT as well as 5 cases of solid AdCC. Interestingly, the authors found that AdCC-HGT carried fewer copy number changes compared with solid AdCC. This is in contrast to the findings by Seethala et al,[59] where chromosomal gains were mostly confined to the areas of HGT, whereas losses were seen only in the conventional areas.

AdCC-HGT is a biologically aggressive tumor, and hence it displays a high frequency of locoregional recurrence and metastasis to lymph nodes and distant sites.[60] The rate of metastasis to lymph nodes is ~57% compared with only 5% to 25% in patients with conventional AdCC.[12] The high propensity for lymph node metastasis, which is at least 5 to 10 times higher than for conventional AdCC, strongly supports neck dissection for patients with this unique histology.[60] Taking into account the possibility that some or even many of reported hybrid salivary tumors of various types may actually represent HGT, the number of cases of AdCC-HGT could be even higher than reported in the literature. Of the ~35 cases of hybrid tumors reviewed by Hellquist et al,[61] AdCC was the most common malignancy.

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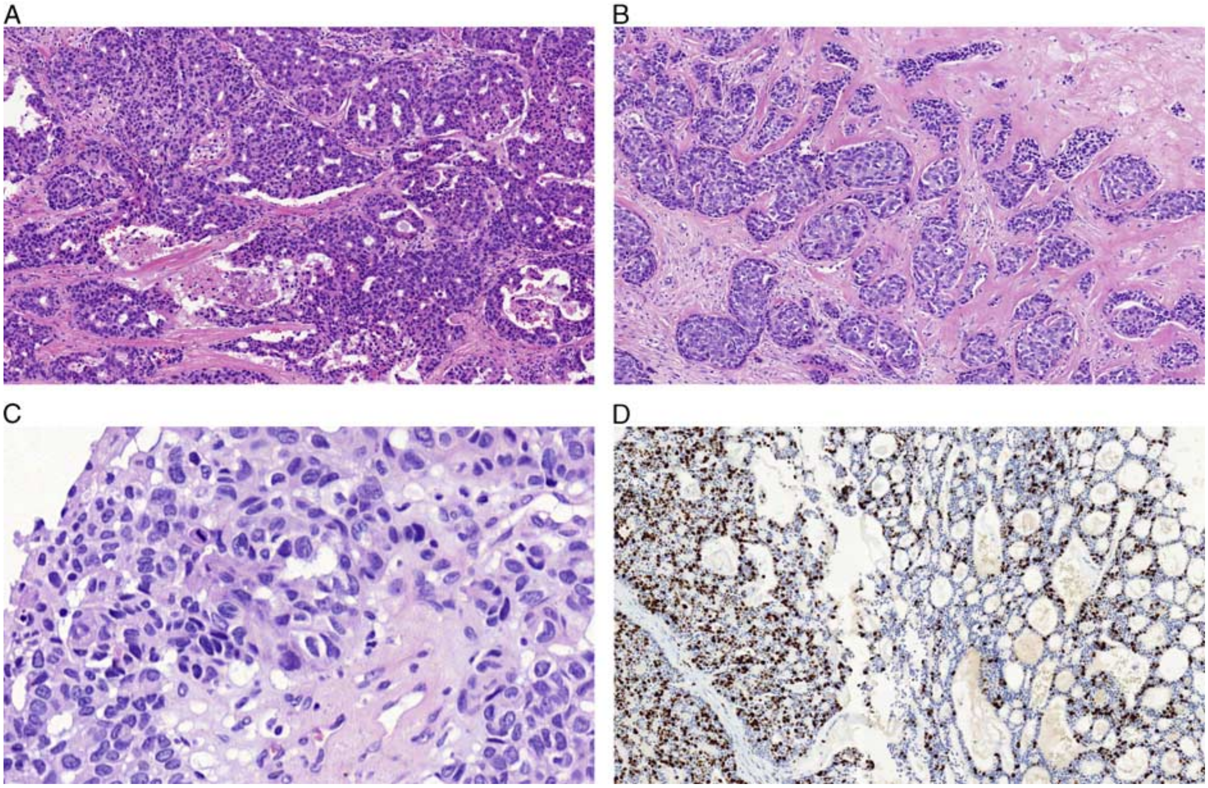

FIGURE 3. High-grade transformation of adenoid cystic carcinoma (AdCC). Low-power view shows 2 distinct carcinomatous components: conventional adenoid cystic carcinoma and high-grade carcinoma with a predominantly solid growth pattern, forming irregular and confluent tumor nests (A, B). High-grade carcinoma component exhibits cells with large pleomorphic nuclei and moderate amount of cytoplasm (C). Ki-67 labeling index of the high-grade carcinoma component (left portion) is much higher than that of the conventional AdCC component (right portion) (D).

Epithelial-Myoepithelial Carcinoma

EMC is a rare salivary gland malignancy of presumed intercalated duct origin comprising about 1% of all salivary gland tumors. The name, first used by Donath et al[62] in 1972, denotes a biphasic tubular neoplasm of clear myoepithelial cells surrounding small lumina lined by ductal epithelial cells. EMC is considered to be an LG malignancy, mainly occurring in the parotid gland, but it may also arise at any minor salivary or seromucous gland site, including the upper and lower respiratory tract.[14]

Histologically, EMC is characterized by a malignant tumor composed of variable proportions of 2 cell types that typically form duct-like structures. The biphasic morphology is represented by an inner layer of duct-lining, epithelial-type cells, and an outer layer of usually clear, myoepithelial type cells. The architecture varies and may include tubular, papillary, cystic, and solid patterns.[14] Areas of myoepithelial overgrowth usually do not show marked atypia or mitotic activity. Factors that have been shown to predispose to recurrences include positive resection margins, vascular invasion, necrosis, and myoepithelial anaplasia.[14]

Epithelial-myoepithelial carcinoma containing an high-grade component (EMC-HGT) has been described under various terms, including "high-grade carcinoma component in epithelial-myoepithelial carcinoma,"[63] "dedifferentiated epithelial-myoepithelial carcinoma,"[14,64–67] "epithelial-myoepithelial carcinoma with HGT,"[68–71] "aggressive epithelial-myoepithelial carcinoma,"[72] and "epithelial-myoepithelial carcinoma with myoepithelial anaplasia."[14,73] Abrupt transition of the myoepithelial and/or ductal component of EMC into HG carcinoma is referred to as "dedifferentiated epithelial-myoepithelial carcinoma," whereas myoepithelial anaplasia was defined by Seethala et al[14] as severe nuclear atypia and pleomorphism that occurs gradually in the myoepithelial component. Histologically, EMC-HGT frequently exhibits solid growth with loss of the characteristic biphasic arrangement of tumor cells (Fig. 4A). The neoplastic cells in the HG areas exhibit classic features of malignancy, including increased nuclear size and pleomorphism, increased nucleolar size, increased mitotic activity, and frequent necrosis. In EMC-HGT, the HG component may display either an epithelial or myoepithelial phenotype, and the HG cells may exhibit an epithelioid, plasmacytoid, clear cell, squamous, or spindle morphology (Figs. 4B, C).[14,69,74] The proliferative activity is always significantly increased in the HG component (Fig. 4D). Interestingly, in 1 recent case, the HG areas exhibited dual ductal and myoepithelial phenotype, manifesting morphologically as salivary duct carcinoma and MC.[67]

EMC-HGT is a biologically much more malignant tumor than its conventional counterpart. The reported survival in EMC-HGT is between 1 and 172 months, whereas for patients with conventional EMC the average disease-free period is > 11 years. In line with this, EMC-HGT has a 50% risk of lymph node and a 30% risk of distant metastasis, as

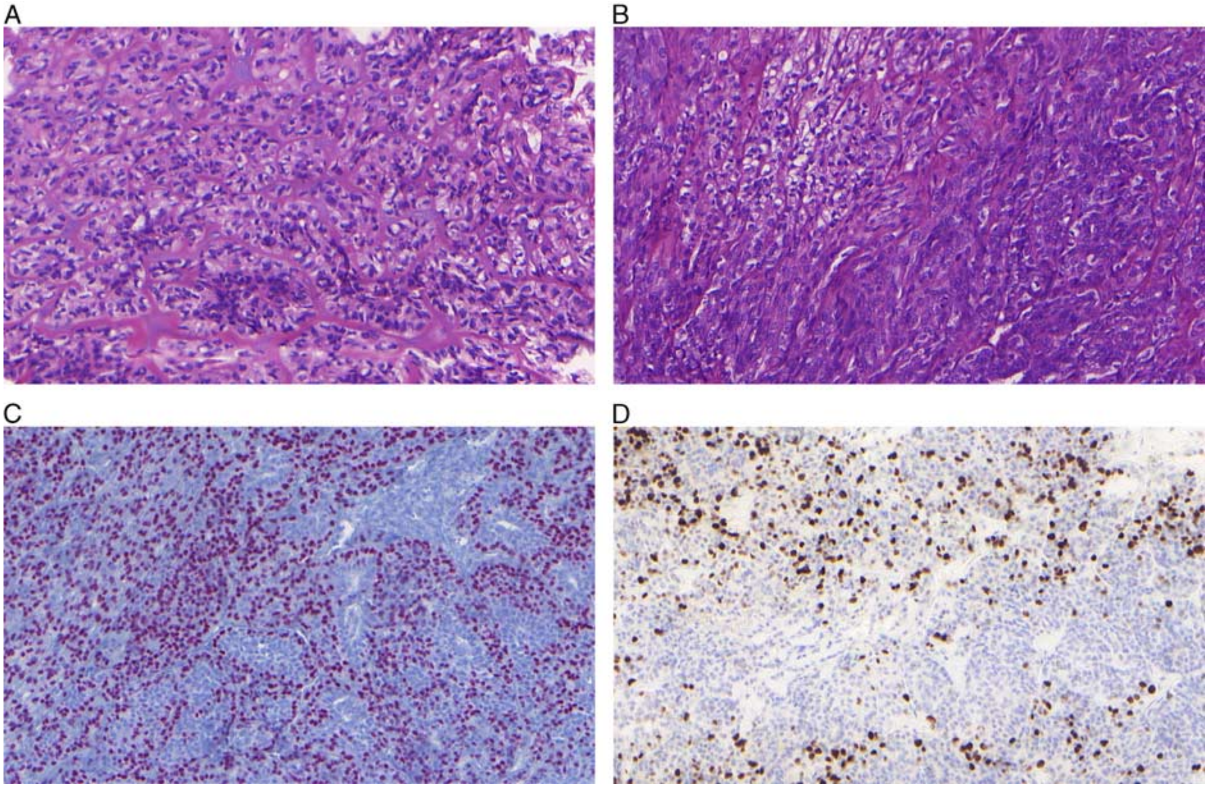

FIGURE 4. High-grade transformation of epithelial-myoepithelial carcinoma. Two distinct carcinomatous components are intermingled; biphasic low-grade epithelial-myoepithelial carcinoma (small lower portion) and monophasic high-grade carcinoma with myoepithelial features (left upper portion) (A). High-grade cells exhibit clear cell and spindle morphology (B). Myoepithelial phenotype is revealed by nuclear immunopositivity for p63 protein (C). Higher Ki-67 labeling index is noted in the high-grade carcinoma component (left upper portion) (D).

reviewed by Roy et al.[69] The average age of patients with EMC-HGT is higher than that of conventional EMC (72 vs. 60 y).[69] Akin to conventional EMC, HGT-EMCs most commonly occur in the major (parotid and submandibular) glands.

Myoepithelial Carcinoma

MC is an underrecognized and challenging entity with a broad morphologic spectrum. Histologically, MC is defined as a tumor composed almost exclusively of myoepithelial cells and characterized by an invasive growth pattern.[75] Among salivary gland malignancies, MC seems particularly challenging, as it is rare and understudied, and the diagnostic and prognostic criteria are poorly defined. MC seems to be relatively aggressive with a high risk of recurrence (cumulative recurrence rate of 43%), including local recurrence (cumulative rate of 35%), lymph node metastasis (cumulative rate of 21%), and distant metastasis (cumulative rate of 22%).[76] Recent studies have shown that MC is a fusion gene-driven malignancy predominantly characterized by gene fusions involving *PLAG1* and less frequently *HMGA2*.[77,78]

There is apparently a spectrum of biological behavior in MC, including cases with HG morphologic criteria in up to 40% of cases.[79–83] Nevertheless, HGT in MC has been described in only 1 case of LG MC by Ogawa et al.[81] The neoplasm displayed an undifferentiated carcinomatous phenotype without evidence of myoepithelial differentiation comprising ~20% of the tumor volume. The malignant cells of the HG component were polygonal and spindled featuring significant nuclear pleomorphism and the presence of atypical mitotic figures. In the immunohistochemical study, malignant cells in the HG component overexpressed p53 and cyclin D1 (not detected in conventional MC). The proliferative activity was 60% in the HG component compared with 10% in the LG MC. Despite aggressive therapy, there were local recurrences at 5 and 9 months. The recurrent tumor was composed of undifferentiated carcinoma with anaplastic spindle cells growing in fascicular arrangements.[81]

Mucoepidermoid Carcinoma

HGT is an extremely rare event in MEC with only 3 documented examples.[82–84] In 2003, Nagao et al[82] reported the first case in a 55-year-old man, involving the parotid gland. The second case was a tumor arising in the bronchus of an 11-year-old girl.[83] The patient reported by Nagao and colleagues had 2 local recurrences at 3 and 7 months but did not develop metastases during a 10-year follow-up. In contrast, the pediatric patient died 3 months after diagnosis and had developed metastatic disease to the pleura, mediastinal lymph nodes, abdominal wall, and vertebral bones. Histologically, the HG component in the case by Nagao and colleagues was the predominant part of the tumor and was labeled as anaplastic "undifferentiated" carcinoma. This HG component featured solid and sheetlike growth with no glandular or cystic components. The authors described focal sarcomatoid areas and extensive necrosis. The HG malignant cells exhibited large pleomorphic nuclei with conspicuous nucleoli and a high mitotic rate. There were no mucous or squamous cells present. Nagao et al[82] reported that the HG component was aneuploid, whereas the conventional MEC was diploid. In the pediatric case, there was a mutation of TP53, selectively located in the cells of the HG component. A corresponding immunohistochemical overexpression of p53 was also documented.[83]

The *CRTC1-MAML2* fusion gene is a highly specific diagnostic marker for MEC, and it is reported in most LG and intermediate-grade MECs, while fewer HG MECs are fusion-positive,[83–88] and indeed the fusion-negative HG MEC–like neoplasms may not actually be true MECs.[86,88] Thus, identification of this fusion gene could also be useful for diagnosing a "dedifferentiated" MEC. The first case of MEC-HGT with a *MAML2* break apart fluorescence in situ hybridization (FISH) study was reported recently in the minor salivary glands of a 73-year-old woman.[84] The resected tumor histologically consisted of 2 carcinomatous components. Approximately 30% of the tumor showed a conventional MEC, while 70% comprised an HG transformed component. In the conventional MEC component, FISH revealed a *MAML2* rearrangement. Also, the HG transformed cells showed multiple split signals interpreted as a *MAML2* rearrangement and subsequent polyploidization. The tumor was removed with a wide resection plus neck and retropharyngeal lymph node dissection followed by adjuvant radiation therapy. Nevertheless, the tumor grew rapidly, new metastatic lymph nodes were revealed, and the patient died 7 months after diagnosis.[84]

Polymorphous Adenocarcinoma

PAC,[89] previously known as PLGA,[90] is defined as a malignant epithelial tumor characterized by architectural diversity, cytologic uniformity, an infiltrative growth pattern, and a low metastatic potential.[91] After MEC, it is the second most common type of malignant neoplasm of minor salivary glands.[92] Histologically, PAC typically contains a single tumor cell type arranged in cords, single file, trabeculae, or tubules swirling or concentrically layered around nerves or vasculature. In general, PAC is considered as an LG carcinoma with an overall recurrence rate of 19%, a risk of regional lymph node metastasis of 13% to 17%, and a low rate of distant metastasis.[93,94] In 1999, however, Michal et al[95] described the cribriform adenocarcinoma of the tongue (CAT). In the original series, CAT occurred exclusively in the tongue, had an infiltrative lobular growth pattern and prominent cribriform, solid, or glomeruloid architecture, and was associated with a high risk (over 60%) of lymph node metastases.[95] Subsequently, the same authors

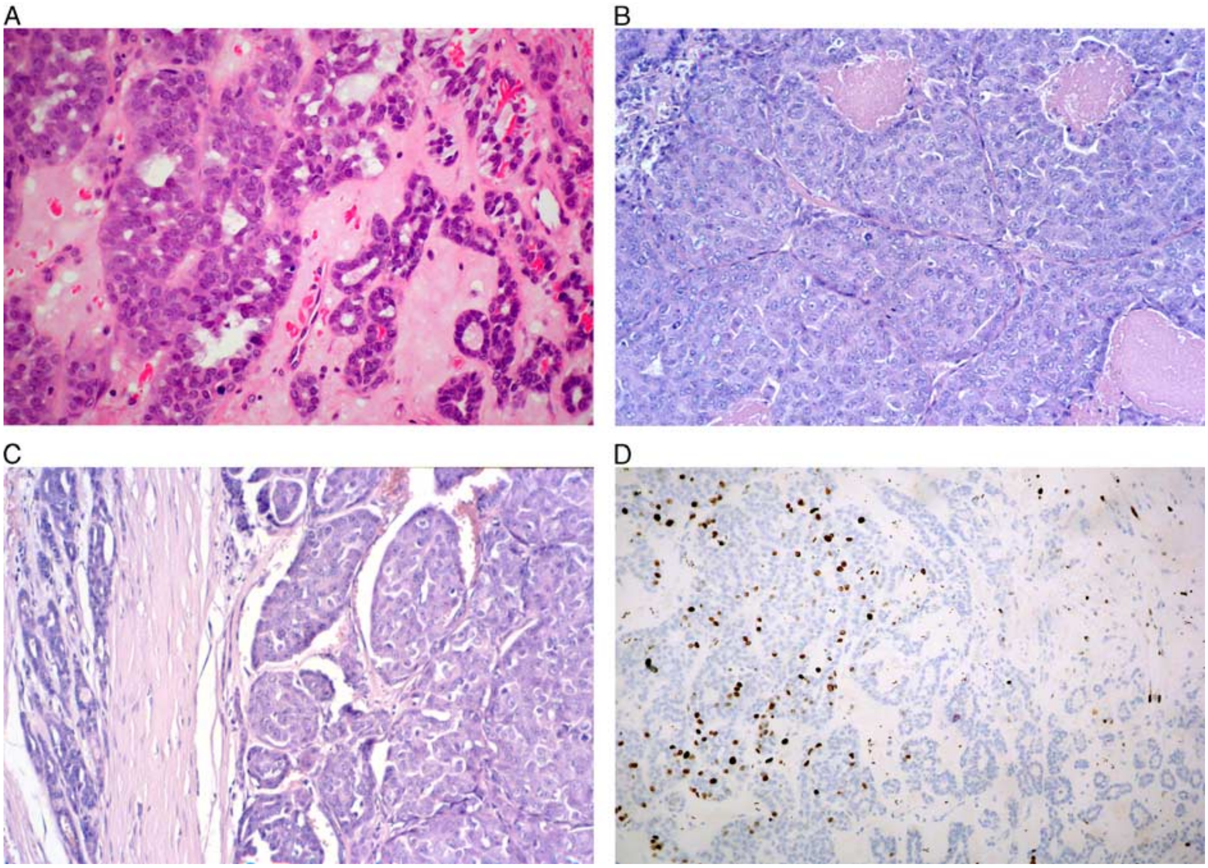

FIGURE 5. High-grade transformation of polymorphous carcinoma. The tumor is composed of 2 distinct components. One is consistent with conventional low-grade polymorphous adenocarcinoma (right portion), while the other component is poorly differentiated carcinoma (left portion) (A). The latter exhibits a high-grade morphology characterized by a predominantly solid growth pattern, nuclear atypia with prominent nucleoli, foci of necrosis, and high mitotic count (B, C). Ki-67 proliferative activity is increased in the high-grade component (D).

reported identical neoplasms at other minor salivary gland sites, modifying the diagnostic term to cribriform adenocarcinoma of the salivary gland (CASG).[96] The ability reliably to distinguish PAC and CASG is not irrelevant as several other studies have shown that CASG is associated with a high rate (up to 72%) of lymph node metastasis[95–97] compared with 10% to 17% rate in classic PAC.[93,98] Similarly, in a more recent cohort, the risk of nodal metastases was 6% for PAC, and 31% for CASG.[99]

There are only 6 cases of classic PAC/(PLGA) with HGT reported in the literature (5 occurring in the palate and 1 in the nasal cavity).[15,100–102] These tumors were composed of 2 distinct components. One was consistent with conventional LG PAC, while the other component was considered to be poorly differentiated adenocarcinoma or "undifferentiated" carcinoma. The latter exhibited an HG morphology characterized by a predominantly solid growth pattern, nuclear atypia with prominent nucleoli, foci of necrosis, and high mitotic count (Figs. 5A–C). Ki-67 proliferative activity was increased tenfold in the latter compared with that observed in the conventional PAC component (Fig. 5D).[15] In 1 case, the HG component displayed androgen receptor positivity, thus resembling salivary duct carcinoma.[15] Despite the fact that HGT in many salivary gland tumors is recognized as an event with a more aggressive clinical course, only 1 patient with PAC-HGT in the literature appears to have died as a consequence of the disease.[101] No case of CASG with HGT has been described so far.

Clear Cell Carcinoma

CCC, also known as hyalinizing CCC, is an uncommon salivary gland carcinoma composed of nests and cords of clear cells with monomorphic basophilic nuclei in a background of a heavily hyalinized stroma.[103] CCC exhibits a consistent *EWSR1* rearrangement in FISH analyses as a result of the *EWSR1-ATF1* fusion oncogene.[104] CCC is an LG malignancy with a propensity to occur in the minor salivary glands and with a very low risk of locoregional lymph node and distant metastasis. The neoplastic cells have bland nuclear features, low mitotic activity, and moderately abundant clear cytoplasm, and they tend to infiltrate surrounding tissues embedded in a desmoplastic and hyalinized collagenous stroma.

There have been only 2 examples of CCC-HGT described in the literature, both arising in the base of the tongue.[17,105] Reportedly, the first case showed small foci of HG morphology (nuclear anaplasia, high mitotic activity, and necrosis) within an otherwise typical CCC.[105] The authors called this case an "aggressive variant" of CCC, but according to the description, the case meets the criteria for HGT.[98] Despite the limited extent of the tumor with HG morphology, the patient developed widespread metastases and died within 1 year.[98] The second case was composed of "sheets of clear and eosinophilic cells with a background of a myxoid-like matrix" containing areas with large, bizarre malignant tumor cells, focal necrosis, and atypical mitotic figures.[17] A rearrangement of *EWSR1* was identified in a FISH study. The tumor was diagnosed as poorly differentiated carcinoma, not otherwise specified. More typical features of CCC including the *EWSR1* gene rearrangement were revealed in a FISH analysis of a recurrent tumor 10 months after radiation therapy. The patient was confirmed to have locally recurrent disease and pulmonary metastases.[17]

Summary

In conclusion, HGT/dedifferentiation in salivary gland tumors seems to be a more complex process than a simple progression through histologic grades. Currently, the most useful tool in identifying a transformed component is still a combination of morphologic criteria aided by Ki-67 analysis. Although p53 abnormalities have been demonstrated in the transformed component of AdCC, the molecular genetic mechanisms responsible for HGT in most salivary gland carcinomas remain largely unknown. Salivary carcinomas with HGT represent good candidates for genetic profiling for precise subtyping but also for detection of secondary genetic alterations of potential therapeutic relevance such as HER2 overexpression. In cases with potential therapeutic targets in the HG component such as those with HER2 overexpression, a biopsy of the metastatic disease might be of help to verify that the metastases are derived from the HG clone and not the LG component. HGT in salivary carcinoma is associated with aggressive clinical behavior and a usually poor prognosis irrespective of the histologic type of the original tumor. A high propensity for cervical lymph node metastasis suggests the need to include neck dissection in the therapy. HGT in salivary gland carcinoma can occur either at initial presentation or less commonly at the time of recurrence, sometimes following the administration of postoperative radiotherapy. In practice, the potential for the presence of HGT in almost any type of salivary gland carcinoma warrants a thorough sampling of all salivary gland tumors to avoid missing a HG component.

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