Case Report

Primary cutaneous neuroendocrine tumor (atypical carcinoid) expressing KIT and PDGFRA with myoepithelial differentiation: a case report with immunohistochemical and molecular genetic studies

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Abstract: Primary cutaneous neuroendocrine tumors (NET) except for Merkel cell carcinoma have rarely been reported. Herein reported is a very unique case of primary cutaneous NET with immunohistochemical markers of myoepitheliomas. A 47-year-old woman presented a tumor measuring 0.8x0.9x0.6 cm of the face. The tumor was excised completely with wide margins. Morphologically, the tumor was located in the dermis, and the tumor was composed of epithelioid cells arranged in trabecular, sinusoidal, rosette, ribbon-like, and cord-like patterns. Focal areas show tubular formations. The tumor cells were homogenous, and their nuclei showed hyperchromasia but no apparent histological features of malignancy were seen. The stroma was very scant. No invasive features were seen. Immunohistochemically, the tumor cells were strongly positive for cytokeratin (CK) 34BE12, CD5/6, CK14, NCAM (CD56), p63, and KIT (CD117), and moderately positive for CK AE1/3, p53, chromogranin, synaptophysin, neuron-specific enolase (NSE), PDGFRA, CA19-9, and Ki-67 antigen (labeling index=23%). The tumor cells were negative for CK CAM5.2, CK7, CK8, CK18, CK19, CK20, EMA, vimentin, CEA, HMB45, S100 protein, α-smooth muscle antigen, desmin, CD34, GFAP, neurofilaments, CD99 (MIC2), CD45, CD57, ErbB2, TTF-1, MUC1, MUC2, MUC5AC, and MUC6. Mucins examined by d-PAS and Alcian blue techniques were negative. A genetic analysis using PCR-direct sequencing method in paraffin sections identified no mutations of \( \text{KIT} \) (exons 9, 11, 13 and 17) and \( \text{PDGFRA} \) (exons 12 and 18) genes. Imaging modalities including CT and MRI identified no tumor in the body. The clinicians thought that the tumor was cured. She was a sailor and immediately visited other countries; therefore the follow-up could not be done.

Keywords: Skin, NET, carcinoid, neuroendocrine, myoepithelioma, immunohistochemistry, KIT, PDGFRA

Introduction

Primary cutaneous neuroendocrine tumors (NET) are extremely rare. In the WHO blue books of skin tumors [1] and soft tissue [2], there are no descriptions of NET except for Merkel cell carcinoma of the skin [3]. Thus, there are now no descriptions of NET in the skin and soft tissue tumors in WHO blue books. Primary cutaneous NET has been reported as a name of carcinoid [4-7], and there have been only 4 cases of primary cutaneous carcinoid, to the best of the author’s knowledge. Herein reported is a very unique case of primary cutaneous NET (atypical carcinoid) expressing KIT and PDGFRA with immunohistochemical markers of myoepithelial lineage.

Case report

A 47-year-old woman, a sailor, presented a tumor measuring 0.8x0.9x0.6 cm of the face, and consulted our hospital. The tumor was confirmed by doctors, and the tumor was excised completely with wide margins.

Morphologically, the tumor was located in the dermis (Figure 1A), and the tumor was composed of epithelioid cells arranged in trabecular, sinusoidal, ribbon-like, rosette, and cord-like patterns (Figure 1B-E). Focal areas show tubular formations (Figure 1B and 1D). The tumor cells were homogenous, and their nuclei showed hyperchromasia but no apparent histological features of malignancy were seen.
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(Figure 1A-E). Mitotic figures were seen in 3 per 10 high power fields (Figure 1B-E). Apoptotic figures were also scattered (Figure 1B-E). The stroma was very scant. No invasive features were seen (Figure 1A). No lymphovascular permeation was seen. The overall histological diagnosis on the hematoxylin and eosin (HE) sections was atypical carcinoid.

Because cutaneous atypical carcinoid (NET) is extraordinary rare, an immunohistochemical study was done with the use of Dako Envision method (Dako Corp, Glostrup, Denmark), as previously reported [8, 9]. Immunohistochemically, the tumor cells were strongly positive for cytokeratin (CK) 34BE12 (Figure 2A), CD5/6 (Figure 2B), CK14 (Figure 2C), NCAM (CD56) (Figure 2D), p63 (Figure 2E), and KIT (CD117) (Figure 2F), and moderately positive for CK AE1/3, p53 (Figure 2G), chromogranin (Figure 2H), synaptophysin (Figure 2I), NSE (Figure 2J), PDGFRA (Figure 2K), CA19-9 (Figure 2L), and Ki-67 antigen (labeling index=23%) (Figure 2M). The tumor cells were negative for CK CAM5.2, CK7, CK8, CK18, CK19, CK20, EMA, vimentin, CEA, HMB45, S100 protein, α-smooth muscle antigen, desmin, CD34, GFAP, neurofilaments, CD99 (MIC2), CD45, CD57, ErbB2, TTF-

Figure 1. Morphologically of the tumor. The tumor is located in the dermis (A). The tumor is composed of epithelioid cells arranged in trabecular, sinusoidal, ribbon-like, rosette, and cord-like patterns (B-E). Focal areas show tubular formations (B, D). The tumor cells are homogenous, and their nuclei showed hyperchromasia but no apparent histological features of malignancy are seen (A-E). Mitotic figures are seen in 3 per 10 high power fields (B-E). Apoptotic figures are also scattered (B-E). The stroma was very scant. No invasive features are seen (A). No lymphovascular permeation is seen (A-E). The overall histological diagnosis on the hematoxylin and eosin section is atypical carcinoid. HE; A: x40. B: x100. C-E: x200.
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1, MUC1, MUC2, MUC5AC, and MUC6. Mucins examined by d-PAS and Alcian blue techniques were negative. The proportion of neuroendocrine positive cells was 95% in NCAM immunostaining, 35% in chromogranin immunostaining, 30% in synaptophysin immunostaining, and 50% in NSE immunostaining.

Because tumors positive for KIT and PDGFRA proteins may show mutations of KIT and PDGFRA genes [10-17], a molecular genetic analysis of KIT gene (exons 9, 11, 13, and 17) and PDGFRA (exons 12 and 18) gene were performed by the PCR direct sequencing method, as previously reported [10-38]. The exons of

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**Figure 2. Immunohistochemistry.** The tumor cells are strongly positive for cytokeratin (CK) 34BE12 (A), CK5/6 (B), CK14 (C), NCAM (CD56) (D), p63 (E), and KIT (CD117) (F). The tumor cells are moderately positive for p53 (G), chromogranin (H), synaptophysin (I), NSE (J), PDGFRA (K), CA19-9 (L), and Ki-67 antigen (labeling index=23%) (M). A-M: x200.
both genes were selected because they are frequent mutation sites [10-17]. The primers are shown in Table 1. In brief, genomic DNA was extracted from paraffin blocks with proteinase K digestion and phenol/chloroform extraction, and subjected to PCR for 40 cycles (94°C for one minute, 52°C for one minute, 72°C for one minute), using a thermal cycler (GeneAmp PCR system 9700, Applied Biosystems, ABI, CA). The annealing temperature was 53°C. PCR products were extracted, and subjected to a computed automatic DNA sequencer (ABI PRIZM 3100 Genetic Analyzer, Applied Biosystems, ABI, CA).

The retrospective genetic analysis using PCR-direct sequencing method in paraffin sections identified no mutations of KIT (exons 9, 11, 13 and 17) and PDGFRA (exons 12 and 18) genes in the present tumor.

Imaging modalities including CT and MRI identified no tumors in the body. The clinician thought that the tumor was cured. She was a sailor and immediately visited other countries; therefore the follow-up could not be done.

Discussion

The present case is not Merkel cell carcinoma. The HE histologies of the present case are entirely different from Merkel cell carcinoma. Immunohistochemically, the present case was negative for CK20, which was consistently positive and its perinuclear dotted immunoreactivity is very sensitive and specific findings of Merkel cell carcinoma.

The HE histologies of the present case showed typical “carcino- noid” organoid patterns including sinusoidal, trabecular, cord-like, tubular, rosette, and ribbon-like patterns. The tumor cells showed hyperchromasia and mild nuclear enlargement. Mitotic and apoptotic figures were seen, though they were scant in number. The tumor had no invasive features and no lymphovascular permeation is seen. The tumor was completely resected. Although some rippled patterns were seen, the current tumor is not sebaceous carcinoma. Therefore, the HE diagnosis of atypical carcinoid seems correct.

Every tumor (particularly carcinoma) of every organ can show “neuroendocrine differentiation”. Thus, it is frequently difficult or impossible whether tumors with neuroendocrine features should be labeled as “NET” or “tumors with neuroendocrine differentiation”. At the present time, there is no consensus of this differentiation. In NET of lungs, small cell lung carcinoma was defined on only HE histology, and small cell lung carcinoma does not express neuroendocrine features in about 10% of cases [39]. In large cell neuroendocrine carcinoma (LCNEC) of the lung, it is mandatory to demonstrate the neuroendocrine features, but the percentage of LCNEC cells with neuroendocrine features is not described in the WHO blue book of lung [40]. However, in WHO blue book of breast [41], NET is defined as a neuroendocrine tumor whose neuroendocrine features are seen more than 50% of the tumor cells.

Therefore, there seem to be an unwritten consensus that tumors with neuroendocrine cells > 50% of tumor cells are called “NET”. In contrast, tumors with neuroendocrine cells < 50% of tumor cells are regarded as “tumor with neuroendocrine differentiation”. In the present case, the author examined four pan neuroendocrine markers (chromogranin, synaptophysin, NCAM, and NSE), and found that the proportion

| Table 1. Primer sequence |
|-------------------------|-------------------------|
|             | Forward | Reverse |
| KIT exon 9 | 5'-TCC TAG AGT AAG CCA GGG CTT-3' | 5'-TGG TAG ACA GAG CCT AAA CAT CC-3' |
| KIT exon 11 | 5'-GAT CTA TTT TTC CCT TTC TC-3' | 5'-AGC CCC TGT TTC ATA CTG AC-3' |
| KIT exon 13 | 5'-GCT TGA CAT CAG TTT GCC AG-3' | 5'-AAA GGC AGC TTG GAC ACG GCT TTA-3' |
| KIT exon 17 | 5'-CTC CTC CAA CCT AAT AGT GT-3' | 5'-GTC AAG CAG AGA ATG GGT AC-3' |
| PDGFRA exon 12 | 5'-TTG GAT ATT CAC CAG TTA CCT GTC-3' | 5'-CAA GGG AAA AGC TCT TGG-3' |
| PDGFRA exon 18 | 5'-ACC ATG GAT CAG CCA GTC TT-3' | 5'-TGA AGG AGG AGT AGC CTG ACC-3' |
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of neuroendocrine positive cells was 95% in NCAM immunostaining, 35% in chromogranin immunostaining, 30% in synaptophysin immunostaining, and 50% in NSE immunostaining. Therefore, the present tumor fulfills the criteria of “NET” [41].

The present cutaneous tumor appears primary skin tumor, because imaging techniques revealed no other tumors in the body. Immunohistochemically, the negative TTF-1 suggests that the tumor is not a metastasis from lung carcinoma. The CK7-/CK20- pattern is compatible with primary cutaneous tumor.

Characteristically, the present tumor showed that the tumor had high molecular weight CK such as CK34BE12 and CK5/6, and did not have low molecular-weight CK such as CK CAM5.2, CK7, CK8, CK18, CK19, and CK20. CK AE1/3 showed moderate reaction. In addition, interestingly, the present tumor showed CK14, which is mainly expressed in basal cells or myoepithelial cells of the prostate, breast, salivary glands and other organs. The present tumor also strongly expressed p63, a satellite molecule of p53. The p63 labels many cell types, and it is one of the markers of basal cells and myoepithelial cells [42]. Thus, the positive expression of CK34BE12, CK5/6, p63, and CK14 may suggest that the present tumor may have myoepithelial characteristics. Recently, myoepithelioma or myoepithelial carcinoma has been reported in the skin [43-45], soft tissue [46] and other organs [47, 48]. The cutaneous myoepithelial neoplasms are linked to mixed tumor (chondroid syringoma) [43-45]. However, these myoepithelial tumors most commonly express other myoepithelial markers including S100 protein, α-smooth muscle antigen, desmin, and GFAP [43-47], all of which were negative in the current tumor. This suggests that the current tumor is not myoepithelial tumor but shows myoepithelial differentiation. Although the author recently demonstrated that cutaneous basal cell carcinoma (BCC) frequently express neuroendocrine antigens, KIT and PDGFRA [49], the present case is not BCC histologically and immunohistochemically. Of interest is that myoepithelial carcinoma can express KIT and PDGFRA [47], which were seen in the present study.

Of particular interest is that the current tumor expressed KIT and PDGFRA. Both are transmembranous receptor tyrosine kinases, and their mutations are profoundly involved the malignant nature of the some tumors such as gastrointestinal stromal tumor (GIST), e-GIST, melanoma, germ cell tumors, hematopoietic malignancies and other tumors [10-17]. It is well known that NET frequently expresses KIT and PDGFRA [18-38]. The author also has reported a case of carcinoid of the pleura expressing KIT [50]. Therefore, there is a tendency that KIT and PDGFRA are expressed in NET. The present study also investigated mutations of the genes of KIT and PDGFRA, and found no mutations in the exons examined. In general, NEC including carcinoid [50] expressing KIT and PDGFRA does not have mutations of the two genes of KIT and PDGFRA [18-38]. The present NET (atypical carcinoid) expressed KIT and PDGFRA, but no mutations were seen in both genes. These are new findings.

The present study performed a wide range of immunohistochemistry. The expression of p53 in the present case suggests that the p53 gene mutations are present in the current tumor. The current tumor showed relatively high Ki-67 labeling index (23%), indicating relatively high cellular proliferation fractions. These findings strongly suggest that the tumor is not an ordinary NET (carcinoid) but a NET (atypical carcinoid) with malignant potential. However, the present NET is not neuroendocrine carcinoma (NEC), which shows much more atypical cells and more p53 and higher Ki-67 labeling index.

The expression of CA19-9 is a curious phenomenon. CA19-9 is usually expressed in normal and neoplastic epithelium of the gastrointestinal tract. The expression may suggest that the present NET has focal glandular differentiation.

EMA was negative in the present tumor. EMA was expressed mainly epithelial cells, but EMA-negative epithelial cells are often recognized. The negative EMA does not exclude the epithelial nature of the current tumor. Vimentin was negative in the current tumor, indicating that the tumor is not mesenchymal tumor. CEA was negative, indicating that the current tumor is not adenocarcinoma. HMB45 and S100 protein were negative in the present tumor, indicating that the current tumor is not malignant melanoma. The negative expressions of S100 protein α-smooth muscle antigen, desmin, and
GFAP suggest that the myoepithelial characters are weak in the present tumor, as described earlier. Neurofilaments and CD99 (MIC2) were negative in the current case, suggesting that the current tumor is not Ewing/PNET tumor. CD45 and CD57 were negative, suggesting that the tumor is not lymphoid tumor. ErbB2 was negative, suggesting negative ErbB2/FGF signaling pathway in the current tumor. TTF-1 was negative, suggesting that the tumor is not primary lung tumor. MUC1, MUC2, MUC5AC, MUC6, and mucins were negative, suggesting that the present tumor do not show glandular differentiations.

In conclusion, the author reported an extraordinary rare case of primary cutaneous NET (atypical carcinoid) with immunohistochemical and genetic analysis.

**Conflict of interest statement**

The author has no conflict of interest.

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