Collision Tumor of Primary Laryngeal Mucosal Melanoma and Invasive Squamous Cell Carcinoma with IL-17A and CD70 Gene Over-Expression

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Abstract The most common primary malignancy of the larynx is the squamous cell carcinoma (SCC). The primary malignant melanoma is quite rare in this location. Less than 60 cases of laryngeal melanomas have been reported to date. To our knowledge, collision primary malignant melanoma and invasive squamous cell carcinoma in the vocal cords has not been reported. We report a 53-year-old male patient who was diagnosed with a collision tumor of laryngeal melanoma and invasive SCC. Multiple Th17 pathway related genes including CTLA-4, IL-17A-F, PLZF, FoxP3, Ror γ T, CD27, and CD70 were analyzed by reverse transcriptase-polymerase chain reaction (Rt-PCR) in this case. Both IL-17A and CD70 genes were detected in this case of collision tumor. The results may define useful biomarkers for early diagnosis of mucosal melanoma and open an immunotherapeutic field for clinical management with the potential benefit from the immunomodulators that enhance both genes.

Keywords Collision tumor · Laryngeal mucosal melanoma · Squamous cell carcinoma · IL-17A and CD70 genes

Introduction

Approximately 40,000 new patients are diagnosed with head and neck cancers each year in the United States, and squamous cell carcinoma represents more than 90% of all cases [1]. Squamous cell carcinomas account for 95% of primary laryngeal tumors. Most patients are in the fifth, sixth, or seventh decades of life at the time of diagnosis, with an average age of about 60 years. Smoking and alcohol abuse are by far the principal risk factors [2]. Mucosal melanomas represent 1.3% of all cutaneous and non-cutaneous melanomas [3]. Among mucosal melanomas, 55% are located in the head and neck region [3], involving the nose and paranasal sinuses, followed by the oral cavity [4]. Rarely, primary melanomas arise in the larynx. In fact, less than 60 cases of laryngeal melanomas have been reported to date [4, 5].

The pathogenesis of mucosal melanoma is unknown. Several speculated risk factors include exposure to sunlight, human papilloma virus, chronic irritants, and carcinogenic compounds [4]. Recently, several studies reported that malignant melanomas were related to an altered immune system. Several TH17 related genes have been studied in cutaneous melanoma, including CTLA-4, IL17, ROR γ t, and FoxP3, with very limited studies done in mucosal melanomas. These genes are of interest for immunomodulator development [6].

The term “collision tumor” indicates two coexistent but independent tumors with distinct morphology. Collision tumors affecting larynx are extremely rare. To the best of

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our knowledge, a laryngeal SCC coexisting with a primary laryngeal mucosal melanoma has not previously been described.

We report a 53-year-old male patient who was diagnosed with collision tumor of laryngeal melanoma and invasive SCC, with over-expression of IL-17A and CD70 genes, by reverse transcriptase-polymerase chain reaction (Rt-PCR).

Case History

A 53-year-old man presented to the ear, nose and throat (ENT) clinic complaining of a 6-month history of hoarseness. He also had pain in his left throat and odynophagia. He denied dysphagia or dyspnea. He reported a 25-pound weight loss over the past 3 months. He had smoked half a pack of cigarettes per day for 15 years. He is also a hepatitis C carrier. Physical examination did not demonstrate any cutaneous pigmented lesion suspicious for malignant melanoma. Computed tomography (CT) of head and neck revealed a nodular enhancement along the anterior 2/3 of the left true vocal cord and an enlarged left subclavicular

lymph node measuring 1.6 cm in the greatest dimension (Fig. 1a). Laryngoscopic examination revealed a tan–blue thin plaque on the left side of the larynx (Fig. 1b). The lesion extended from the left false vocal cord to the true vocal cord along the anterior 2/3 of the cord. The remaining areas of larynx were clinically unremarkable. The lesion was biopsied and the diagnosis was laryngeal mucosal melanoma. Four weeks later the patient underwent a total laryngectomy (Fig. 1c) and left selective neck dissection.

Postoperatively the patient recovered and was discharged without any complications. Subsequently he received high dose radiation therapy. He denied any complication from the treatment. He has been followed up at the clinic on a regular basis for 15 months. There is no evidence of recurrence or distant metastases to date.

Pathological Findings

Microscopic examination of biopsied samples demonstrated laryngeal squamous mucosa with proliferation of pleomorphic heavily pigmented, rounded, and dendritic melanocytes in the epithelial basal layer and in the upper levels of the squamous epithelium (Fig. 2a). There were melanocytes in the lamina propria admixed with heavily pigmented macrophages. The melanocytes exhibited enlarged nuclei, and some of them had basophilic nucleoli. Confirmatory immunohistochemical studies by standard ABC-Biotin immunoperoxidase (Dako, CA) were performed. The tumor cells were positive for S-100 protein, HMB-45, Melan-A, and PNL-2. The immunohistochemically positive melanocytes were distributed in the epithelium and the lamina propria. Atypical squamous cells were noted as well. A PAS stain demonstrated pseudophyphae of *Candida* in the cornified layer. The biopsy was diagnosed a laryngeal mucosal melanoma. Gross examination of the total laryngectomy specimen revealed a 2.5-cm ulcerated, black, nodular area located on the left laryngeal mucosa, extending from the false vocal cord to the true vocal cord (Fig. 1c). Microscopic examination revealed sheets of heavily pigmented, round-to-plump melanocytes infiltrating in the submucosa, presented as single cells and in small nests along and above the basal layer of the epithelium (Fig. 2b–d). The melanocytes were highlighted by immunopositivity for HMB-45 (Fig. 2e) and Melan-A. The depth of melanoma was 2.8 mm. In addition, there was markedly dysplastic squamous cells in the epithelium (Fig. 2b–c) and submucosa (Fig. 2d), consistent with in situ and invasive squamous cell carcinoma admixed with atypical pigmented melanocytes (Fig. 2b–c). The final diagnosis was mucosal malignant melanoma and invasive SCC of the larynx. The resection margins were uninvolved by tumor. No

Fig. 1 **a** Coronal section from non-contrast CT scan shows a 1.7-cm mass present at the left vocal cords, filling the ventricular space (arrow). **b** Endoscopic view shows a bluish and light brown mass on the left larynx (arrow). **c** Total laryngectomy specimen shows a dark pigmented mass involving the left vocal cords (arrow)

Fig. 2 Low magnification from the left vocal cord mass shows a laryngeal mucosal tumor composed of dark pigmented cells (a). A closer view shows the over-lined squamous mucosa with high grade dysplasia and pigmented atypical melanocytes (b–c). Invasive squamous cell carcinoma infiltrating into submucosa with mixed atypical melanocytes (d). Immunostain for HMB-45 highlights melanocytes in squamous epithelium and submucosal area (e)

angiolymphatic invasion was noted. No metastatic disease was identified in six lymph nodes.

Molecular Findings

Formalin fixed paraffin tumor tissue was analyzed for the genes CTLA-4, IL-17A, IL-17B, IL-17C, IL-17D, IL-17E, IL-17F, PLZF, FoxP3, Ror γ T, CD27, and CD70. RNA was extracted with TRIzol after deparaffinization with xylene and first strand cDNA synthesis was performed. PCR for HGAPDH was used as an internal control to normalize samples. Nested PCR for these genes was performed. Agarose gel electrophoresis of the amplicons showed that IL-17A and CD70 were detected (Fig. 3).

Discussion

It is well known that more than 95% of primary laryngeal malignancy is SCC. Most patients are in the fifth, sixth, or

Fig. 3 PCR amplicons. Appropriate sized bands were detected for IL-17A and CD70. No bands were found for IL-17B, IL-17D, IL-17F, PLZF, CD27, CD70, FoxP3, Ror γ T and negative control (no template DNA). A 100 bp ladder is shown in the left lane. HGAPDH is used as an internal control for reverse transcription

seventh decades of life at the time of diagnosis, with an average age of about 60 years [1]. Smoking and alcoholic factors are contributed to the development of laryngeal

SCC. Other risk factors that have been proved, suggested, or debated include prior radiation exposure, HPV infection, genetic factors, air pollution, dietary deficiencies, chronic gastrointestinal reflux, and occupational exposure to wood or metal dusts and asbestos, etc. [2].

Mucosal malignant melanoma is infrequent. The head and neck region accounts for more than half of all mucosal melanoma cases, the rest are distributed in the anus and rectum, the female genitalia, and the urinary tract [4, 7]. Mucosal melanoma of the head and neck area most commonly occur in the nasal cavity, followed by the oral cavity [8]. Primary laryngeal melanomas are rare, with less than 60 reported cases to date. Most primary laryngeal malignant melanomas occur in elderly male patients, in their sixth or seventh decades of life [5, 9, 10]. The majority of the patients are white with only two reports of Asian individuals [10, 11]. In contrast to laryngeal melanoma, gender predilection is not observed in cutaneous melanoma patients. And most cutaneous melanoma patients are younger, in their fourth and fifth decades of life [9].

Patients can present with various symptoms including sore throat and dysphagia, but the most common presenting symptom is hoarseness. Tumors typically involve the supraglottic larynx for primary laryngeal melanoma. The second most common site is the glottis [5, 9, 10]. Risk factors of laryngeal melanoma are unclear, but most studies noted a strong association between a history of cigarette smoking and laryngeal melanomas [5, 9]. Our patient was a long-term smoker who also developed a concurrent laryngeal invasive squamous cell carcinoma for which smoking is a well-documented risk factor.

A collision tumor represents the coexistence of two morphologically distinct tumors. A primary squamous cell carcinoma of the larynx has been reported to occur simultaneously with other laryngeal neoplasms. Tomidokoro et al. reported an isolated case of collision tumor of primary laryngeal SCC and leiomyosarcoma [12]. The term “double tumor” has been suggested by Medina-Banegas et al. for their case report of synchronous chondrosarcoma and epidermoid carcinoma in situ of the larynx [13]. In our current case, it is important to distinguish between primary laryngeal melanoma and metastatic malignant melanoma in the larynx. Some authors suggest that the diagnosis of primary laryngeal melanoma can not be made if lacking the in situ melanoma component [9, 14, 15]. Although, it has also been hypothesized that melanoma in the larynx may arise from the submucosal melanocytes and melanocytes from the mucoserous gland [16]. We also believe that primary laryngeal melanoma has to show a melanoma in situ component. Additionally, the diagnosis should be supported by the lack of melanoma elsewhere in the body. Therefore, the diagnosis of primary laryngeal melanoma may not be made solely by exclusion of other primary sites.

Morphologically, melanoma is a notorious mimicker, which can appear to be epithelioid, spindled, and plasmacytoid. It can arrange in an organoid pattern or a solid growth pattern. Immunohistochemical studies including S-100, human melanoma black-45 (HMB-45), Melan-A, microphthalmia transcription factor (MITF) and PNL-2 can aid the confirmation of mucosal melanoma [17].

Recently, several studies reported that malignant melanomas were related to an altered immune status. Several genes of interest including IL-17, CTLA-4, CD70, and CD27 have been extensively studied for immunotherapy development [18–21]. IL-17E and IL-17A were found to be higher in cell supernatants and blood serum of patients who have oral epithelial squamous cell carcinoma when compared to healthy people [22]. In our study we were able to identify two over-expressed genes including IL-17A and CD70. It is impossible to determine whether these two genes are associated with only SCC or only mucosal melanoma, or both malignancies in this collision tumor.

Th17, a subset of CD4 that produces IL-17, has been implicated in various autoimmune disorders including rheumatoid arthritis and psoriasis. It has been shown to have anti-tumor immunity in a number of studies [18]. At least six members of IL-17 have been identified. It has been shown that IL-17A and IL-17C are able to induce mRNA expression inflammatory cytokines including IL-1 β , IL-16 and IL-23 [19]. IL-23 recently has been demonstrated to be a promising cancer vaccine for melanoma [23, 24]. Speculatively, this patient might carry a favorable prognosis when compared to patients without an over-expressed IL-17A. However, the role of Th17 on tumor has not yet been clearly identified. Th17 has been shown to have different effects on tumor growth. Some studies demonstrated that Th17 can promote the growth of tumors, while other studies showed that it had an antitumor property. It has also been shown that IL-17A can impair immune surveillance by CD8 and promote neovascularization [25]. Perhaps the most important factor of antitumor immunity is the balance of regulatory T cells (T regs) and Th17 in the tumor microenvironment as illustrated in the work of Kryczek et al. [26]. It has been hypothesized that Th17 has a protective property in the early stage of the disease but becomes permissive as the disease progresses [27]. Whether Th17 has protective function for melanoma metastasis is still controversial.

CD70, a tumor necrosis factor-related cell surface ligand, has been extensively studied in the field of immunotherapy and it has been demonstrated to have anti-tumor immunity. Transfection of tumor cells with CD70 enhances antitumor immune response in murine tumor models [21]. Furthermore, Cormary et al. have been able to demonstrate a stronger anti-tumor immune response elicited by CD40L and CD70 expressing B16F10 tumor cells, based on the

fact that both CD40L and CD70 are costimulators needed for full activation of T cells [28]. They showed that the tumor growth slowed down significantly when CD40L and CD70 were coexpressed by the tumor [28, 29]. Therefore, we speculate that our patient should elicit a strong immune response against the tumor resulting in a favorable prognosis based upon his overexpression of CD70 and IL-17A.

Like laryngeal SCC, the mainstay treatment for primary mucosal melanoma in the head and neck region is radical surgery with or without adjuvant therapy including radiation and chemotherapy. Although radical surgery yields the best local control, local recurrence occurs frequently [9]. A 42% recurrence rate has been reported by Snow et al. [30]. Most recurrent cases occur in the nasal cavity [29]. Shah et al. reported a 64% recurrence rate. However, primary laryngeal melanoma has a relatively low recurrence rate. Yet the survival rate of laryngeal melanoma is still low. The 5-year survival rate is less than 20% due to metastatic disease [9, 16].

In summary, we report a 53-year-old male patient with a collision primary laryngeal malignant melanoma and invasive squamous cell carcinoma of the larynx with IL-17A and CD70 over-expression. This gene expression may open the door to the future where individual melanoma patients will be treated with tailored immunotherapies. In addition, this gene expression may serve as useful biomarkers used for early diagnosis and predicting the prognosis. However, more mucosal melanoma case studies with long-term follow-up are necessary for better understanding of the function of these genes in order to apply them in the treatment for mucosal melanomas.

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