Virchows Arch (2000) 437:203–207

CASE REPORT

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Atypical melanocytic proliferation associated with squamous cell carcinoma in situ of the esophagus

Received: 10 December 1999 / Accepted: 6 March 2000

Abstract We present the case of a 64-year-old woman who underwent a transhiatal esophagectomy subsequent to the presence of high-grade dysplasia of the esophageal squamous epithelium in repeated biopsies. In the resection specimen chronic esophagitis and multifocal carcinoma in situ of the squamous epithelium were diagnosed, associated with a diffuse intraepithelial proliferation of melanocytic cells. While melanocytic hyperplasia (melanocytosis) has previously been recognized as an occasional reactive lesion that can accompany esophageal inflammation and invasive squamous carcinoma, the present case was unusual because of its cytonuclear and architectural atypia in the melanocytic cell population, resembling features of a melanoma in situ in the absence of manifest invasive malignant melanoma. The disappearance of the melanocytic lesion during follow-up supports its nonneoplastic nature, however. This case illustrates that 'malignant features' in esophageal melanocytosis should be interpreted with caution.

Key words Esophagus · Carcinoma in situ · Melanocytosis · Melanoma in situ

Introduction

Scattered melanocytes in the epithelio-stromal junction of the esophageal mucosa are an incidental finding. This phenomenon was first described in 1963 by De la Pava, who found melanocytic cells in the esophagus in 4% of his autopsy material [2]. In subsequent studies the incidence of esophageal melanocytes ranged between 2.5% and 8% [10, 13, 14, 16].

Not surprisingly, primary melanocytic lesions in the esophagus are a rarity. Endoscopically, melanocytic disorders may occur as pigmented mucosal lesions, although this is not always the case. Histopathologically, two conditions have been described at this site: esophageal melanocytosis (also termed melanocytic hyperplasia) and (primary) malignant melanoma.

Esophageal melanocytosis is defined as an increase in the number of melanocytes spreading along the junction area [7, 16]. This condition is considered benign and has been described in association with chronic esophagitis, squamous epithelial hyperplasia and infiltrating squamous carcinoma of the esophageal mucosa [10].

In the present report we describe a very unusual form of intraepithelial melanocytic proliferation in the esophageal mucosa. The proliferation of melanocytes was observed throughout the specimen and appeared in association with multifocal carcinoma in situ of the squamous epithelium and chronic esophagitis, thus resembling changes previously described as melanocytosis. However, marked cytonuclear and architectural atypia in this melanocytic cell population fulfilled the criteria of neoplastic change, and the differential between reactive versus neoplastic melanocytic proliferation needed consideration. To our knowledge, the present case is the first described of melanocytosis with marked atypia.

Clinical history

A 64-year-old woman was referred to our hospital with a 6-month history of odynophageal complaints. She described retrosternal pain radiating to the neck and back elicited by various foods and drinks. She developed nausea without vomiting. The patient smoked two or three cigarettes per day and occasionally used alcohol in small amounts. Her family history was remarkable for blind-

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ness caused by retinitis pigmentosa, but otherwise her medical history and physical examination were negative. After gastroscopy the patient was initially treated with an H_2-receptor antagonist and a proton pump inhibitor for a sliding hernia with reflux esophagitis, but her symptoms persisted. Subsequently, biopsies were taken during repeated esophageal endoscopies, which all showed high-grade dysplasia of the squamous epithelium and inflammatory changes. Esophageal manometry did not reveal any motility disorder. Endosonography and an additional laryngo-pharyngoscopy were normal.

After 1 1/2 years of close surveillance the patient underwent a subtotal esophagectomy with gastric tube reconstruction. The postoperative course was complicated by anastomotic leakage, but the salivary fistula healed spontaneously. During a follow-up period of 2 years after surgery the patient was well and no specific clinical problems were encountered.

Materials and methods

The surgical specimen, including the dissected lymph nodes, was fixed in a 4% buffered formalin solution. Transmural tissue blocks were taken from the proximal and distal resection margins, and multiple samples were taken along the entire length of the esophagus, including the area of the squamo-columnar junction and the adjacent proximal part of the stomach. This material was embedded in paraffin and processed for routine histology.

All sections were stained with hematoxylin and eosin (HE). In selected sections melanin staining (according to Schmorl), periodic acid–Schiff / Alcian Blue (PAS/AB) and hemosiderin staining were performed.

On selected paraffin sections immunohistochemistry was performed using a standard avidin–biotin complex method with diaminobenzidine as chromogen. Poly- or monoclonal antibodies directed against cytokeratin 8 and 18 (CAM5.2), carcinoembryonal antigen (CEA), S100 protein (S100), MART-1 (Melan-A) constituents of (immature) melanosomes (HMB45), CD68 (KP1), leukocyte antigen L1 (MAC387), CD1a and Ki-67 nuclear antigen (MIB-1) were used.

Material from selected areas was retrieved from paraffin and processed for electron microscopy according to a previously published protocol [15].

Pathologic findings

The surgical specimen consisted of the subtotal esophagus, with a length of 13 cm (after retraction), and the proximal part of the stomach, including seven lymph nodes.

On gross examination the esophageal mucosa exhibited irregular, flat areas with a rough surface, spreading along the lower half of the esophagectomy specimen. There was no visible pigmentation. Apart from scattered mucosal petechiae, the gastric part of the specimen, including the area of the squamo-columnar junction, appeared normal.

Microscopy revealed multifocal carcinoma in situ of the esophageal squamous epithelium. There was no epithelial dysplasia in the proximal resection margin. Areas of nonneoplastic epithelium adjacent to the dysplastic lesions showed varying degrees of chronic esophagitis. All seven lymph nodes were free of malignant cells.

While these findings confirmed the observations made in several preoperative biopsies, the mucosa of the resection specimen additionally exhibited a population of previously unrecognized large cells, which were diffusely spread throughout the esophageal mucosa with strictly intraepithelial localization, appearing as single cells in some parts but otherwise arranged in small clusters situated in epithelial lacunae, thereby reaching the upper third of the epithelial wall close to the epithelial surface (Fig. 1a, b). These cells exhibited faintly eosinophilic cytoplasm containing focal granular brown pigment, which stained positive in the Schmorl stain for melanin (in the absence of specific stain for hemosiderin; Fig. 1d). The nuclei were medium sized, partly hyperchromatic and in several cells contained conspicuous nucleoli (Fig. 1c). No mitotic figures were detected in this cell population, but sporadic cells showed nuclear immunoreactivity for MIB-1, which was consistent with proliferative activity. PAS/AB staining was negative. Horizontal and vertical intraepithelial spread was prominent in areas immediately adjacent to squamous dysplasia, where inflammatory changes were present (Fig. 1a). A considerable number of these cells spread along the squamo-stromal junction and in the basal epithelial cell layer, thus reaching into the proximal esophageal resection margin.

On immunohistochemistry these cells exhibited strong immunoreactivity for CD68 and S100 (Fig. 1e); there was equivocal reactivity for CEA; and no specific reaction was seen with the antibodies CAM 5.2, CD1a, MAC 387, Melan-A and HMB45.

Electron microscopy of these atypical cells revealed numerous solitary melanosomes (Fig. 2), the majority of which were not fully matured; none was found in lysosomes. Granules and ‘flaps’ indicative of Langerhans cells were not seen.

Based on morphology, marker pattern and ultrastructure, a diagnosis was made of squamous cell carcinoma in situ and chronic esophagitis associated with atypical intraepithelial melanocytic proliferation of uncertain malignant potential.

Repeated biopsies taken during the 2-year postoperative follow-up period showed disappearance of the intramucosal melanocytes consistent with a reactive nature of the lesion.

Discussion

In the normal human esophagus, scattered melanocytes may occur in the epithelio-stromal junction. In 1963, De la Pava was the first to describe pre-existent esophageal melanocytes, which he found in 4 out of 100 autopsies and which were not related to esophageal disorders [2]. Since then, several investigators have confirmed this observation, and in the literature estimates of the prevalence of esophageal melanocytes range between 2.5% and 8%. These data are based on the Japanese population [10, 13, 14, 16].

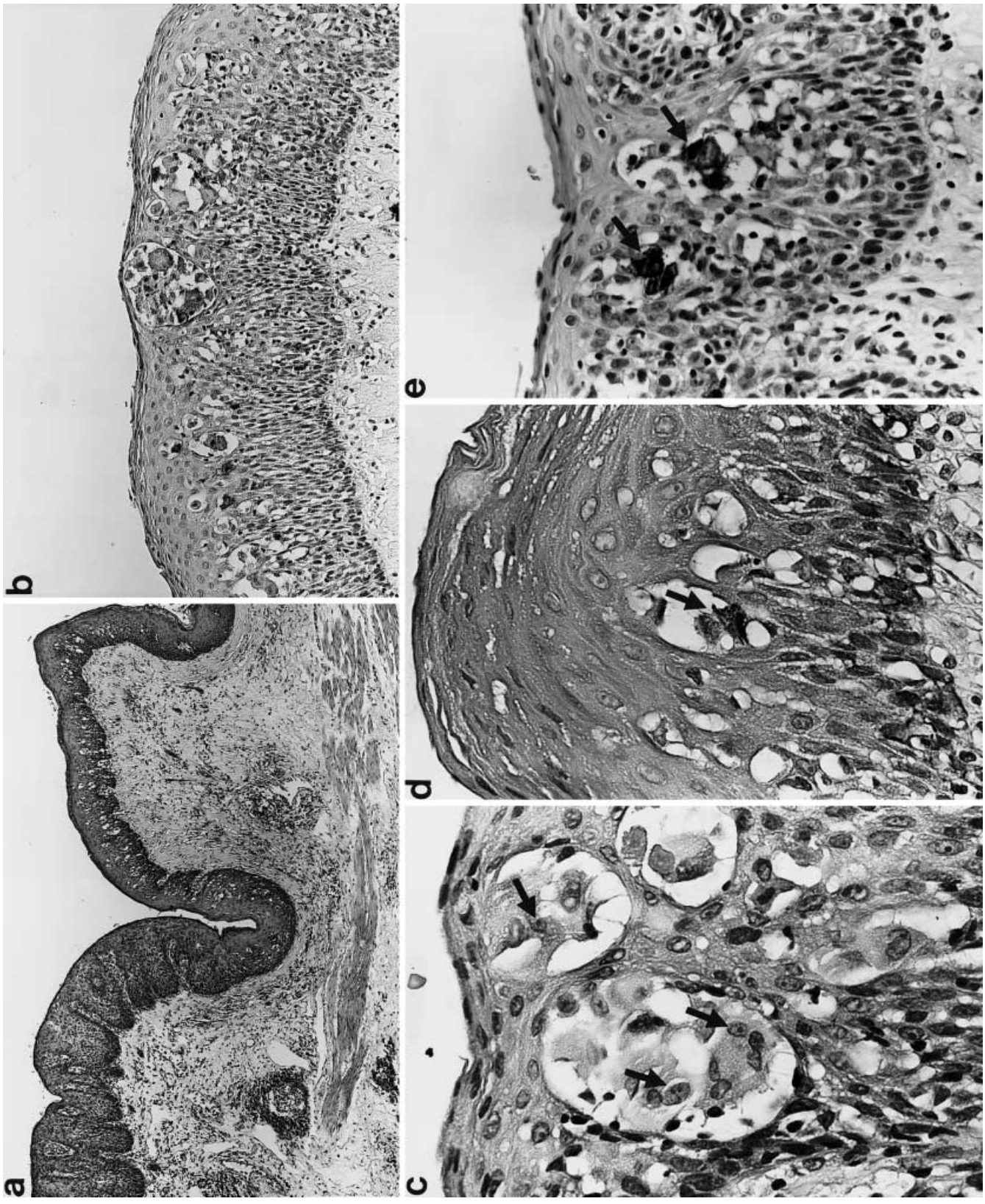

Fig. 1a-e Atypical melanocytic cell proliferation in squamous epithelium of the esophagus. **a** Overview of the esophageal mucosa with high-grade dysplasia of the squamous epithelium (*left*) and adjacent intraepithelial atypical melanocytic hyperplasia (*right*). Note chronic inflammatory infiltrate in the underlying stroma. HE stain **b** Photomicrograph with clusters of atypical melanocytes in the squamous epithelium. HE stain **c** Some atypical cells exhibit conspicuous nucleoli (*arrows*). HE stain **d** Atypical cell with intracytoplasmic granular deposition staining for melanin in the Schmorl stain (*arrow*). **e** Atypical cells staining for S100 (*arrows*). S100 immunohistochemistry, section counterstained with hematoxylin

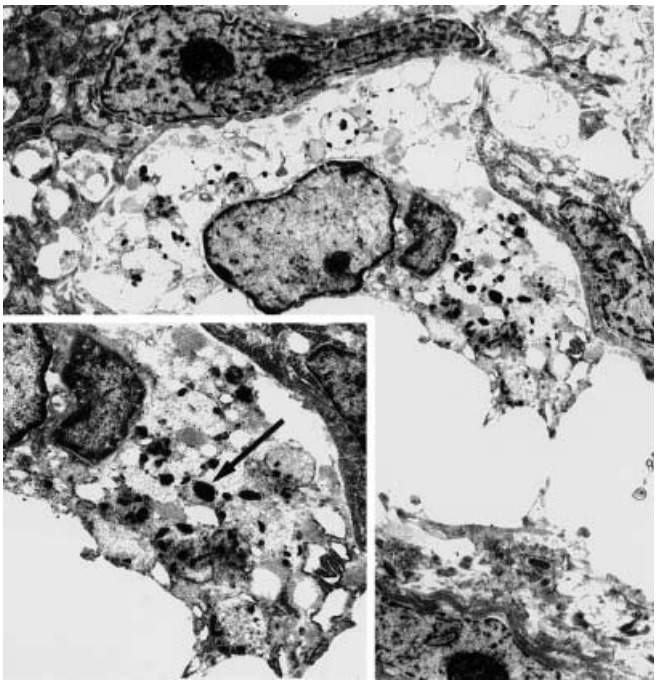

Fig. 2 Electron microscopy of the lesion described, showing the presence of (pre-)melanosomes (*arrow*). ×4,700; *insert* ×7,500

While in the normal esophagus the number of intramucosal melanocytes is low and their presence can easily be overlooked, there are pathologic conditions of the esophagus that are characterized by conspicuous melanocytic hyperplasia. This phenomenon is described as melanocytosis, which refers to an increase in the number of melanocytes in the *epithelio-stromal junction without cytonuclear and architectural atypia* [7]. It is plausible to assume that melanocytosis is based on a proliferation of pre-existing melanocytes in the interface between epithelium and underlying stroma, but this cell proliferation might also arise from scattered stromal neuroectodermal cells, such as Schwann cells, or might even develop from pluripotent epithelial stem cells [10].

As an isolated benign melanocytic disorder in the esophagus, melanocytosis is a rare observation with an estimated incidence of about 0.07–0.15% in patients undergoing endoscopy [3] (see also [9, 11, 16]).

Results of a recent study [10], however, indicate that melanocytosis is much more frequently seen in association with reactive changes of the squamous epithelium, such as acanthosis and basal cell hyperplasia in the setting of chronic esophagitis. Moreover, melanocytosis seems to be a more common phenomenon in surgical esophagectomy specimens removed for carcinoma than in unselected autopsy material, which supports the notion that squamous epithelial dysplasia and carcinoma may develop on the basis of long-standing inflammatory irritation of the mucosa. The fact that melanocytic hyperplasia is observed in the scar tissue of 8% of skin re-excision specimens after initial biopsy for nonmelanocytic benign or malignant lesions might indicate that melanocytic proliferation is part of a general tissue reaction pattern in response to noxious stimuli [4].

At first glance, the melanocytic origin of the lesion described above may appear doubtful, and a pagetoid spread of epithelial cells might be considered; there was positivity for S100 and CD68 and epithelial markers, and PAS/AB were negative. However, electron microscopy unequivocally showed the presence of (pre-)melanosomes in these cells, which is consistent with their melanocytic origin, and the Schmorl staining for melanin pigment was also positive.

As melanocytosis can be one of the mucosal changes observed as a consequence of events finally leading to neoplastic transformation of the epithelium, the question arises as to whether the hyperplastic melanocytic cell population itself may be prone to malignancy. Although hyperplastic melanocytes are plausible candidates as precursor cells for primary esophageal malignant melanoma, the results in the published cases of melanocytosis do not unequivocally point to a causal association between these two conditions. Melanocytic hyperplasia with intraepithelial proliferation of atypical melanocytes exhibiting features of an in situ melanoma would represent a histopathological link between melanocytosis and melanoma, but it is difficult to find convincing evidence for this hypothesis in the literature. Widespread melanocytic hyperplasia has been observed in the presence of a primary malignant melanoma of the esophagus, but these diffuse melanocytic proliferations lacked atypia [12]. When atypia is present in diffusely spread intraepithelial melanocytes, this is closely related to an adjacent primary malignant melanoma, which can exhibit considerable horizontal pagetoid spread [8]. In one case, melanocytosis was reported to precede the occurrence of malignant melanoma metachronously [6]. Various degrees of melanocytic atypia including features of melanoma in situ were reported in a single case of diffuse melanocytic hyperplasia throughout the esophagus, but also this lesion was associated with multifocal invasive malignant melanoma [5]. To our knowledge there are no reports of an association between melanocytosis of the esophagus and the occurrence of retinitis pigmentosa.

The present case is very unusual, as marked cytonuclear and architectural atypia is present in a melanocytic proliferation otherwise characteristic of melanocytosis in the absence of invasive melanoma but associated with squamous cell carcinoma in situ and chronic esophagitis. This observation shows that melanocytosis can indeed present with atypical features to a degree and extent suggestive of neoplastic proliferation, although definitive evidence for a malignant transformation of this melanocytic cell population could not be demonstrated and follow-up biopsies showed disappearance of the lesion.

According to the clinical and experimental experience with the biological behavior of cutaneous atypical melanocytic proliferations classified as lentigo maligna, only a fraction of these lesions seem to develop into invasive

melanoma after many years [1]. This formed the rationale for the further management of this patient, who did not undergo a re-resection but remained under close surveillance including regular endoscopic follow-up. The disappearance of the melanocytic proliferation supports its nonneoplastic nature. It demonstrates that ‘malignant features’ in esophageal melanocytosis should be interpreted with caution.

Acknowledgements The authors thank Prof. Dr. W. Mooi, Department of Pathology, Dijkzigt Hospital, Rotterdam, The Netherlands and Prof. Dr. S.R. Hamilton, Department of Pathology, M.D. Anderson Cancer Center, Houston, Texas for their valuable comments. The help of W. Meun, Department of Pathology, Academic Medical Center, Amsterdam, in preparation of the photographs is greatly appreciated.

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