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REVIEW PAPER

# Conjunctival Melanoma - Epidemiological Trends and Features

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## Abstract

Conjunctival melanoma is a rare but sight and life threatening malignancy. It accounts for 2%–5% of all ocular tumours and 5%–7% of all ocular melanomas with an incidence of 0.2–0.8 per million in the Caucasian population with rare cases reported in the non-Caucasians. In recent decades the incidence of uveal melanoma has been relatively stable whilst conjunctival and cutaneous melanoma have shown increasing incidence which may be connected to the result of environmental exposure to ultraviolet light. The dissimilarity in incidence between light and dark pigmented individuals observed in conjunctival melanomas compared to uveal and cutaneous melanomas may be related to differences in their histological structures and genetic profile. Recent molecular biological studies support the fact that each type of melanoma undergoes its own molecular changes and has characteristic biological behaviour. Further studies are required for each type of melanoma in order to ascertain their individual etiology and pathogenesis and based on this knowledge develop relevant preventative and treatment procedures.

**Keywords** Conjunctival melanoma · Epidemiology · Clinical features · Diagnosis · Management · Prognosis

## Introduction

Conjunctival melanoma is quite a rare but sight and life threatening malignancy with an associated mortality of up to 30% [1–5]. It accounts for 2%–5% of all ocular tumours and 5%–7% of all ocular melanomas with an incidence of 0.2–0.8 per million in the Caucasian population with only rare cases reported in the non-Caucasians [1–21] (Table 1). Recent studies indicate that the incidence of conjuncitval melanoma is increasing [10, 12, 13] and showing a trend similar to that seen in cutaneous melanoma [12, 15]. Conversely, the incidence of ocular and mucosal melanomas is considered to be stable [14]. Although some studies describe an equal incidence in men and women [10, 20, 22], others have reported a slightly higher frequency in male patients [8, 12].

Incidence of conjunctival melanomas increases with age and is found to be more common in older individuals. The mean age at presentation is between 55 to 65 years whilst it is extremely rare in those younger than 20 [1–12, 15, 21, 23, 24]. Recent papers show only 29 reported cases of conjunctival melanoma in children younger than 15 years [21, 24].

Conjunctival melanoma most commonly arise from primary acquired melanosis (PAM) with atypia, estimating approximately 53 to 75% of cases. It can also develop from conjunctival nevus or can be formed de novo without any preceding lesion which has been seen in 18 to 30% of tumours [17, 22, 25]. Only about 5% of conjunctival melanomas are associated with a conjunctival nevus. However, although they are relatively common the greater majority do not progress to a malignant form [1–4, 22].

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**Table 1** Incidence of conjunctival melanomas

| Study group | Incidence rate per 100.000 person-years men/women | Study size | Time period | Country | Comments |
|---|---|---|---|---|---|
| Lommatzcsch et al. [6] | 0.08 | 196 | 1960.–1985. | German Democratic Republic | No time trend in incidence |
| Seregard and Kock [7] | 0.024 | 45 | 1969.–1991. | Sweden | |
| Norregaard et al. [5] | 0.052 | 42 | 1960.–1980. | Denmark | |
| Chang et al. [14] | / | 216 | 1985.–1994. | USA | 4.8% of all ocular melanomas |
| Tuomaala et al. [15] | 0.054<br>M:F ratio did not differ statistically | 85 | 1967.–2000. | Finland | Increasing incidence rates (0.4 to 0.8)<br>Standardised to Finland population |
| Vajdic et al. [9] | 0.08/0.04 | 37 | 1996.–1998. | Australia | Age standardised to Australian population |
| Inskip et al. [16] | 0.036/ 0.022 | 167 | 1974.–1998. | USA | Increasing incidence rates with borderline significance for male<br>Age standardised to USA population |
| Yu et al. [12] | M: 0.018–0.063<br>F: 0.022–0.032 | 206 | 1973.–1999. | USA | Increasing incidence rates for male<br>Age standardised to USA population |
| Missotten et al. [17] | 0.05 | 194 | 1950.–2002. | Netherland | |
| Isager et al. [18] | 0.04/0.03 | 120 | 1943.–1997. | Denmark | 5% of all ocular melanomas<br>Age standardised to world standard population<br>No significant time trend |
| Hu et al. [19] | 0.018 Blacks<br>0.017 American Indians<br>0.015 Asians<br>0.033 Hispanics<br>0.049 non-Hispanic Whites | 168 | 1992.–2012. | United States | Increasing incidence rate |
| Triay et al. [10] | M: 0.01–0.074<br>F: 0.006–0.045 | 170 | 1960.–2005. | Sweden | Increasing incidence rates for both sexes |
| Park et al. [20] | 0.012 | 90 | 1999.–2011. | Korea | No increase in incidence rate |
| Larsen et al. [45] | 0.036 (1960.–1969.)<br>0.087 (2000.–2012.)<br>0.05 (1960.–2012.)<br>0.053/0.048 | 138 | 1960.–2012. | Denmark | Increasing incidence rate |

M: men; F: women

## Clinical Features and Presentation

Conjunctival melanoma usually presents as an asymptomatic raised pigmented plaque, macule or tumour. The size can range from a tiny lesion of less than a millimetre in thickness to large tumour masses encompassing the entire surface of the eye and measuring more than 10 mm in thickness [1–4, 22]. The additional clinical features signifying melanoma include large tumour size, diverse appearance, lack of mobility in relation to the sclera, extension onto the cornea and the presence of large feeder vessels [23]. The colour can range from light to dark brown and only in rare cases these tumours are amelanotic. Although it can appear on any part of the conjunctiva, the most common location of the lesion is the bulbar conjunctiva close to the limbus [25] in which the corneal epithelium is often affected. Other locations, including forniceal and palpebral conjunctiva, plica semilunaris and caruncula are less common however are associated with a less favourable outcome [1–4, 22].

Conjunctival melanomas can grow and spread locally on the eye surface including the cornea as well as spreading directly into the globe or orbit, nasolacrimal system and sinuses [22, 26–28]. They can further metastase via lymphatic and hematogenous broadening. First clinical metastases usually occurs in the regional lymph nodes [22, 29] with medially placed conjunctival melanoma showing a tendency to spread to submandibular, while laterally placed tumours show a predisposition to the preauricular lymph nodes. However distant metastases can occur without prior regional disease [17, 28, 29] with frequent sites being the lungs, brain, liver, skin and gastrointestinal tract [17, 22, 25, 28]. In contrast, the more frequent uveal melanomas metastasize almost solely hematogenously with the most common site being the liver [1–4, 22].

## Histopathology and Staging

Conjunctival melanomas arise from melanocytes located in the basal layer of the epithelium of the conjunctival membrane. They show invasion of atypical melanocytes from the overlying conjunctival epithelium penetrating the basement membrane into the subepithelial connective tissue (substantia propria) [30].

Histopathologic evaluation is used to confirm clinical diagnosis of conjunctival melanoma however, this type of melanoma is not always easy to diagnose. The histopathological classification can be based on tumour thickness and invasion of the substantia propria. Accordingly, conjunctival melanomas can be divided into four groups: (1) melanoma of the conjunctiva confined to the epithelium; (2) melanoma of the conjunctiva with not more than 0.5 mm in thickness, with invasion of the substantia propria; (3) melanoma of the conjunctiva with a thickness between 0.5 to 1.5 mm, with invasion of the substantia propria; and (4) melanoma of the conjunctiva greater than 1.5 mm in thickness, with invasion of the substantia propria [30, 31]. Since tumour thickness is a valuable prognostic factor for regional and distant metastases as well as mortality it is important to make histological sections vertical to the epithelial surface [15, 17, 32, 33]. The thickness of the tumour can be measured objectively with a calibrated microscope and includes distance from the epithelial surface to the deepest extent of the tumour in the substantia propria [22, 26].

The hystopathologic confirmation of conjunctival melanoma diagnosis is reliant on the identification of atypical melanocytes whose appearance vary from apparent to almost undetectable [30, 34]. These cell alterations manifest with pleomorphic nuclei, large nuclear size, prominent nucleoli, mitotic activity with atypical mitoses and abundant cytoplasm [30]. Other histological features used to correlate with clinical findings in order to confirm the diagnosis of malignancy include: pagetoid growth, radial extension of the intraepithelial component, patchy or bandlike inflammation of the basal layer of the lesion, decreased maturation of basal cells and invasion of sclera or cornea [3, 23].

Conjunctival melanoma can be composed of four cell types: small polyhedral cells, large round epithelioid cells, balloon cells, and spindle cells which may present in various proportions [17, 23, 30]. Spindle cells are elongated with large hyperchromatic nuclei and eosinophilic nucleoli [3, 35]. Balloon cells have a larger, rounder morphology, centrally placed nuclei with changed melanogenesis leading to a number of clear vacuoles in the cytoplasm that apply pressure on the nuclei [3, 36]. Small polyhedral cells have clear cytoplasm, pronounced nucleoli and uniformly stained nuclei [3, 37]. Epithelioid cells consist of large round or oval nuclei, prominent nucleoli, marked nuclear pleomorphism, abundant and eosinophilic cytoplasm [23]. Tumours composed of small polyhedral cells can sometimes be very difficult to diagnose and may be incorrectly recognized as a nevus. In these cases constitutional features including cell growth in nests or in a sheet-like fashion can be helpful [22]. Certain histologic features may be beneficial in the prognosis of conjunctival melanoma. Epithelioid cell type is correlated with higher morbidity [2]. Melanomas composed of entirely spindle cell tend to be less aggressive and associated with a more favourable prognosis than mixed cell type lesions. Pagetoid growth, mitotic figures greater than five per high powered field and an absence of inflammatory response may be associated with a poorer prognosis [3, 32].

Diagnosis based on histological features alone can sometimes be difficult and in these cases, immunohystochemical findings can be of value. In making an accurate diagnosis of conjunctival melanoma immunohistochemical studies detecting melan-A, S-100 and HMB-45 may be helpful [22, 30]. Recently, Bcl-2, an anti-apoptotic cell death protein has shown to be a more forceful and reliable immunohistochemical marker for melanocytic tumours of the conjunctiva than S100, Melan-A and HMB-45. Ki-67 can also be of use in determining biological behaviour where lesions positive for Ki-67 are more clinically aggressive. Although immunohistochemistry cannot be fully relied upon in differential diagnosis between benign and malignant conjunctival lesions a combination of both immunohistochemistry and other morphological features can provide a more comprehensive diagnosis of conjunctival melanoma [4, 38].

Conjunctival melanoma may be classified according to the seventh edition of the American Joint Committee on Cancer (AJCC) Tumour, Lymph node and Metastasis (TNM) classification system [30]. This clinical staging is based on tumour size and location of the primary tumour (T), the presence of regional lymph nodes (N1) and distant metastases (M1). The AJCC divides melanomas clinically into tumours of bulbar conjunctiva (T1), non-bulbar (caruncular, palpebral and forniceal) conjunctiva (T2), malignant conjunctival melanoma with local invasion into the globe, eyelid, nasolacrimal system, orbit or sinuses (T3) or invasion into the brain (T4). Furthermore, T1 and T2 tumours can be subdivided according to the number of quadrants of affected conjunctiva. In this classification system the new term "conjunctival melanoma *in situ*" (Tis) is added. Tis represents a form of melanoma restricted to the conjunctival epithelium, formerly known as PAM with severe atypia [21, 30]. Recent studies have shown a correlation between the latest TNM classification system and survival particularly regarding local recurrence [39–41].

## Differential Diagnosis

In differential diagnosis primary consideration should be given to conjunctival nevi, which usually arises in childhood or adolescence, most commonly in the bulbar conjunctiva and

caruncle [1–3, 29]. Further, differential diagnosis includes squamous cell carcinoma and conjunctival squamous intraepithelial neoplasia which are usually amelanotic, but can acquire pigmentation and be similar to conjunctival melanoma [1–4, 23, 26, 30]. Rare lesions which can resemble conjunctival melanoma are staphylomas, subconjunctival haematomas, foreign bodies and haematic cysts, epibulbar extension of uveal melanoma, melanocytoma and metastatic cutaneous melanoma. An accurate diagnosis requires a total excisional biopsy with hystopathological analysis. Incisional biopsy may be unsafe since it can cause seeding of tumour cells in the case of melanoma and should be avoided if possible in order to minimize this risk [1–4, 22, 26, 27].

## Diagnostic Procedures

Conjunctival melanoma is suspected in any adult patient with a new onset pigmented lesion on the surface of the eye. In the event of such changes patients should undergo slitlamp examination of all the conjunctival surfaces (including eversion of the upper eyelid and tarsus) as well as dilated fundus examination. A complete physical examination with the preauricular, postauricular, parotid, submandibular and cervical lymph nodes palpation should also be done. The use of slitlamp photography is recommended in order to record all conjunctival surface changes. Intraocular invasion can be evaluated by gonioscopy, goniophotography and ultrasound imaging. Recently a number of noninvasive imaging techniques have also become available namely ultrasound biomicroscopy (UB), in vivo conofocal microscopy (IVCM) and anterior segment optical coherence tomography (AS-OCT) which can additionally enhance diagnostic accuracy and assist in surgical planning [1–4].

Surgical excision with hystopathology examination will further confirm the diagnosis. In the case of suspected tumour spread into the orbit or metastasis into the brain, lungs and liver additional radiologic imaging such as computed tomography (CT), magnetic resonance imaging (MRI) or ultrasound are advisable. The long term benefit of sentinel lymph node biopsy (SLNB) to track local metastasis has not yet been defined [3, 29, 42].

Patients with established conjunctival melanoma should be referred to an oncologist and monitored two or three times a year in order to detect possible metastatic disease. This assessment should comprise of a complete ophthalmological as well as physical examination with palpation of the head and neck lymph nodes. Annual chest radiography, liver ultrasound scans, liver function tests, brain MRI and abdominal /chest CT imaging are also advisable. Although the role of positron emission tomography (PET) is still uncertain, [1–4, 42] whole-body PET/CT has been reported to be superior to routine radiographic imaging modalities in detecting systemic metastasis, specifically bone involvement, however its use is limited by high cost, false negative results and nonspecificity [3, 27]. Since patients with ocular melanoma are prone to develop cutaneous melanoma and dysplastic nevi regular dermatological examination is also desirable [22].

## Management

The preferred management of all resectable conjunctival melanoma is total surgical excision with “tumour-free” margins of at least 2 to 4 mm combined with supplemental cryotherapy to the remaining conjunctival margins and alcohol corneal epitheliectomy for corneal involvement [1–4, 22, 25, 27]. The aim of these supplemental treatments is to destroy clinically undetectable tumour cells that may persist along the margin of resection and thus prevent dissemination of viable cells [25]. Adjuvant cryotherapy is administered to surgical margins with the conjunctiva raised to avoid damage to the sclera [1–4]. Alcohol epitheliectomy allows for careful removal of involved corneal epithelial tumour after the cells have been denaturated [25]. It is important that the Bowman layer remains intact considering that it makes a natural barrier against deep tumour invasion. During the surgery a dry surgical field is also important to avoid tumour cell spreading [3]. Effective treatment of conjunctival melanomas is complicated by a high rate of local recurrence. In order to provide better local control and eradication of tumour cells, surgical excision is usually combined with additional adjuvant therapy including brachytherapy, cryotherapy and topical application of chemotherapeutic and/or immunotherapeutic agents (Mytomicin C, interferon alpha-2-beta) [1–4, 28].

In the case of extensive lesions when total surgical removal of all macroscopic tumours is not possible incisional biopsies may be performed. However, the possible risk of tumour spread should be kept in mind. During the surgery a “no touch” technique is recommended with the use of fresh sterile instruments at each new step of the surgical procedure, again to minimize the risk of tumour seeding [1–4, 22, 27, 29]. Furthermore, care should be taken to ensure minimal specimen manipulation whereby the specimen is placed on a paper mount so as to prevent scrolling since any curling or distortion of the specimen may alter the diagnosis. Some surgeons do not recommend lamellar sclera excision, which may cause an unattractive scar and may increase the chances of melanoma recurrence in this area with possible intraocular infiltration [27]. Closure is achieved by primary apposition of conjunctiva or applying conjunctival rotational flaps, mucous membrane graft from the contralateral eye or buccal mucosa as well as amniotic membrane transplantation. Amniotic membrane allograft is immunologically well tolerated and provides covering of the exposed sclera as well as having anti-inflammatory and antiscarring properties which help in the healing process and epithelisation, preventing fibrosis, symblepharon and consequent diplopia as well as inhibiting angiogenesis [3].

In some cases in order to restructure the fornix, deepening sutures or a symblepharon ring is required. In the case of diffuse, lateral, intra-epithelial spread or underlying PAM, additional topical chemotherapy with mitomycin C or immunotherapy with interfernon alpha-2b in some cases is administered. Residual or recurrent PAM is treated with excision, cryotherapy or topical mitomycin C [1–4, 29, 42]. Brachytherapy is recommended even if histology suggests complete surgical clearance since conventional sectioning may have overlooked the deepest part of the tumour. In cases where the tumour involves the fornix or caruncular area proton-beam radiotherapy should be administrated [27]. Local recurrence is common in conjunctival melanoma, with a reported incidence ranging from 36 to 62% [1–4, 22, 29]. Surgical excision alone without adjuvant therapy has been shown to be a prognostic factor for local relapse [1–4, 22, 28]. Lesions that involve the globe necessitate a modified enucleation while those that extend into the orbit require orbital exenteration [25, 27].

The use of SLNB for the purpose of staging of the disease and identifying patients with micro nodal metastasis is controversial [27, 42]. It has been advocated in melanomas with a high risk for local metastases, such as those more than 10 mm in diameter and 2 mm in thickness and in nonlimbus location [1–4, 17, 27, 42]. Patients with initial lymph node metastases have a better prognosis than those with initial systemic metastases [22]. Once systemic metastatic disease have been indentified further treatment requires chemotherapy unfortunately usually with poor prognosis [1–4, 22].

## Prognosis

Despite the fact that conjunctival melanoma is s rare disease it has serious implications with respect to recurrence, metastasis and mortality with location and tumour extension being among the most significant prognostic factors [3]. Unfavourable locations include palpebral and forniceal conjunctiva, plica semilunaris, carunculae and lid margins. These locations are associated with a higher risk for local recurrence in more than 50% of these cases as well as a higher mortality [15–28]. The presence of one or more recurrences is associated with an increased incidence of distant metastases [28]. Melanoma arising de novo is also associated with a higher risk of metastases and death compared to those arising from nevus and PAM [28, 29]. An additional risk factor for recurrence is reported to be surgical excision without adjunctive treatment [25, 27] thus routine administration of adjunctive therapy irrespective of histological clearance significantly diminishes the recurrence rate [1–4, 27, 43].

Histopathological findings of mixed cell, atypical melanocytes, full-thickness epithelial involvement, absence of inflammatory response and lymphatic invasion by tumour cells is associated with higher mortality [28]. Nodular growth pattern of the tumours, positive margins on histopathology findings, increasing tumour thickness (more than 2 mm), increasing diameter and tumour-associated lymphangiogenesis carries increased risk for local recurrence, lymphatic spread, distant metastases and melanoma-related death [1–4, 17, 25–27]. Patients with regional periocular metastases which can be clinically identified and histologically confirmed also have a poor prognosis with subsequent systemic metastases in most cases [1–4, 22].

## Metastases and Survival

Metastases in conjunctival melanoma as in cutaneous melanoma occur via lymphatic and hematogenous spread. Metastatic disease develops in 20–30% of patients [1–4, 22, 25, 27, 30]. There is compelling circumstantial evidence correlating local-tumour recurrence with metastasis [1–4, 22, 25, 27]. However distant metastases can occur without prior regional disease [17, 42]. It is noteworthy that up to 50% of the patients with systemic metastases show regional lymph node involvement prior to systemic disease development [27, 42]. Conversely, 26% of patients with regional metastases never develop systemic disease [22, 27, 28, 42]. In 45 to 60% of cases, the first sites for metastasis are the regional lymph nodes, predominantly in the parotid and preauricular as well as in submandibular and cervical region [27, 29]. Systemic disease most commonly involves the lungs, brain, liver, skin, bones and the gastrointestinal tract [22, 25, 27, 28] and occurs in approximately 16% after 5 years and 26% of patients after 10 years [1–4, 17, 25]. The survival rate for conjunctival melanoma ranges from 74 to 86% after 5 years and 41 to 78% after 10 years [1–4, 13, 15, 17, 25, 42, 44]. Data obtained from five studies including 649 cases showed that the average frequency of melanoma-related deaths after surgical resection with tumour-free margins was 18% (114/649) over a mean interval of 4.9 years [3].

## Genetic Abnormalities in Conjunctival Melanoma

Extensive studies related to genetic mutations in cutaneous melanoma as compared to those originating in other extracutaneous sites have been conducted. Additionally in recent years the knowledge pertaining to genetic mutations underlying conjuctival melanomas has also increased [27, 45]. There is a growing body of evidence demonstrating a common genetic kinship between conjunctival and cutaneous melanoma, while also differentiating it from uveal melanoma [36, 46–53] (Table 2).

Conjunctival melanomas show mutation of *BRAF* (7q34) [26, 46, 48]. This gene encodes a serine/threonine kinase in the mitogen-activated protein kinase (MAPK) pathway, which is involved in signal transduction [27]. Using multiplex ligation-

**Table 2** Genetic differences between cutaneous, uveal and conjunctival melanoma

| | Conjunctival melanoma | Uveal melanoma | Cutaneous melanoma | Activity |
|---|---|---|---|---|
| BRAF | Present in 14–50% | Not detected | Present in 32–60% | Serine/threonine kinase mutation which inhibits apoptosis and promotes cellular proliferation |
| NRAS | Present in 0–18% | Present in 0–1% | Present in 15–28% | GTPase whose mutation promotes unregulated cell division |
| KIT | Present in 0–11% | Present in 2.2% | Present in 2–10% | Receptor tyrosine kinase which promotes cell survival and growth |
| NF1 | Limited data | Present in 0–1% | Present in 14% | NF1 mutations determine MAPK pathway activation |
| GNAQ<br>GNA11 | Not detected<br>Not detected | Present in 28–50%<br>Present in 32–58% | Present in 2%<br>Present in 4–7% | GNAQ and GNA11 encode a heterotrimeric GT-binding protein $\alpha$-subunit<br>Mutations in GNAQ and GNA11result in constitutive activation of MAPK pathway |
| BAP1 | Not detected | Present in 18–58% | Present in 3% | Deubiquitinating hydrolase involved in tumour suppressor activity, DNA damage response and proliferation |
| SF3B1 | Not detected | Present in 10–24% | Not detected | Involved in pre-messenger RNA splicing<br>Alternative splicing results in multiple transcript variants encoding different isoforms. |
| EIF1AX | Not detected | Present in 8–21% | Not detected | Encodes eukaryotic translation initiation factor 1A |
| P16 | Expression similar to cutaneous melanoma | Different expression in comparison to conjunctival and cutaneous melanoma | Expression similar to conjunctival melanoma | P16 is a CDK inhibitor that slows down the cell cycle by prohibiting progression from G1 phase to S phase<br>Acts as a tumour suppressor |
| HSP 90 | Over-expressed compared to benign nevi | Present in 50–68% | Over-expressed compared to benign nevi | Regulate cellular proliferation and differentiation.<br>Anti-apoptotic function playing an important role in the folding, stabilization, activation, aggregation of several client oncoproteins in tumours cells<br>Participates in cancer development and progression. |
| TERT | 32% | Not detected | 33% | A ribonucleoprotein polymerase that maintains telomere ends by addition of the telomere repeat TTAGGG<br>TERT promoter mutations with UV signature (C > T and CC > TT) in conjunctival melanoma suggest an UV-induced pathogenesis and similarity with cutaneous melanoma |
| Chromosome anomalies | Complex karyotype - cannot provide valuable prognosis<br>Gains of 1q, 3p, 7,17q<br>Losses of 9p, 10, 11, 12q | Simple karyotype – has valuable prognostic information<br>Gains of 6p and 8q<br>Losses of 1p, 3, 6q | Complex karyotype – cannot provide valuable prognosis<br>Gains of 1q, 3p, 7,17q<br>Losses of 9p, 10, 11, 12q | |

BRAF: v-Raf murine sarcoma viral oncogene homolog B; NRAS: neuroblastoma v-Ras oncogene homolog; KIT: feline sarcoma viral oncogene v-kit; NF1: neurofibromin 1; GNAQ: guanine nucleotide-binding protein Q polypeptide; GNA11: guanine nucleotide-binding protein alpha-11; MPAK: mitogen-activated protein kinase; DNA: deoxyribonucleic acid; RNA: ribonucleic acid; CDK: cyclin-dependent kinase; BAP1: BRCA1-assocoated protein 1; SF3B1: splicing factor 3B subunit 1; EIF1AX: eukaryotic translation initiation factor 1A; HSP 90: heat shock protein 90; TERT *telomerase reverse transcriptase*

dependant probe amplification (MLPA), the presence of the *BRAF V600E* mutation in 50% of primary and metastatic conjunctival melanomas has been confirmed [26, 46]. This opens up possibilities for testing and predicting responsiveness to chemotherapy treatment in patient who develop metastatic disease [39]. Other genetic alterations have also been identified in conjunctival melanomas. Primary conjunctival melanomas showed amplification of *DKN1A* and *RUNX2* (both 6p21.2). Further, in metastatic conjunctival melanomas, *MLH1* (3p22.1) and *TIMP2* (17q25.3) were frequently amplified whilst *MGMT* (20q26.3) and *ECHS1* (10q26.3) were frequently deleted [27, 48]. In genetic mutation research, BRAF has received the most attention, partially due to a high level of concordance in the BRAF mutation between cutaneous and conjunctival melanoma. However reported genetic mutations in conjunctival melanomas also include KIT and NRAS. KIT gene encodes CD117, a receptor tyrosine kinase involved in growth and survival is commonly found in mucosal and acral melanomas. Mutations of this gene have only rarely been found in conjunctival melanoma, namely in 2.2% of tumours. NRAS is an oncogene in the Ras family that encodes a GTPase which when mutated activates a signal transduction pathway that leads to unregulated cell division [3, 46, 47, 49]. However, the significance of these abnormalities still remains to be established. *R*ecent data suggest that there may be distinct pathways in tumourogenesis in different ethnic groups which is supported by a study of 53 Chinese conjunctival melanoma patients showing KIT mutations in 11% of conjunctival melanomas and a very low prevalence of *BRAF* mutations (8%) [50]. The identification of BRAF, NRAS and KIT mutations in conjunctival melanoma has shown a striking variation from uveal melanoma and highlights the genetic resemblance to cutaneous melanoma [45].

A better understanding of the underlying genetic and molecular abnormalities implicated in the development and progression of conjunctival melanomas will provide a better opportunity for the improvement of potential targeted therapy [3, 28, 51]. In the near future new and advanced knowledge of conjunctival melanoma molecular pathogenesis will translate into novel and more effective systemic therapeutic agents which will accordingly improve the unsatisfactory prognosis of patients with metastatic disease.

## Conjunctival and Cutaneous Melanomas

Notwithstanding shared cellular origin cutaneous, conjunctival and uveal melanomas show differences and similarities in regard to their incidence rate, pattern of metastasis, treatment modality and underlying genetic mutations [1–4, 19, 22, 28, 43, 54, 55] (Table 3).

The incidence of ocular melanoma has remained stable and in fact according to some studies had declined in past decades, which is the apparent trend for uveal melanoma. Alternatively several studies demonstrate that the incidence of conjunctival melanoma particularly in melanomas located in the sun-exposed conjunctiva [10, 12, 13, 15] is increasing. This rise of incidence largely parallels the incidence of skin melanoma [1–4, 13, 22]. Conjunctival and cutaneous melanomas have mutual genetic features particularly relating to BRAF mutations which implicate the similarity in tumorigenesis and clinical behaviour. The results obtained by Larsen et al. suggest that data of clinical trials which included cutaneous melanoma patients may also be relevant for those with conjunctival melanoma [21].

The role of solar ultraviolet (UV) radiation which is a well known risk factor for cutaneous melanoma is still a questionable risk factor for ocular melanoma and consequently the subject of debate [1–4, 16, 22]. The increase in sunlight-related face melanomas which is not recorded in eye melanomas may be attributable to the protection of the interior eye structures by facial constitution itself, by the cornea and the lens and possibly coupled with the increasing use of sunglasses and other appliances for sun protection [9, 16, 22]. It is considered that in conjunction with solar radiation, genetic and other host factors may also play an important etiological role for different types of ocular melanoma [16, 25, 27]. However, which of them has the greater impact is still unclear. Further to which extent conjunctival and cutaneous melanomas have mutual risk factors also needs to be additionally clarified.

## Ultraviolet Radiation and Conjunctival Melanoma

As has already been mentioned several studies demonstrate a statistically significant increase in the incidence of conjunctival melanomas which is largely parallel to the increase in skin melanoma [9, 12, 28]. Conjunctival melanomas are located on the surface of the eye and therefore directly exposed to UV radiation [1–4, 19, 56]. UVB does not reach the pigmented layer of the uveal tract and only small fluencies of UVA reach melanocytes in the ciliary body and the choroid [1–3, 56]. Epidemiological studies have shown a connection between decreasing latitude and increasing incidence of conjunctival melanoma [57] implying that sun exposure has a role in its development [58]. Recent molecular investigations indicate that conjunctival and cutaneous melanomas share common features [46]. Findings of Rivolta et al. [59] support the occurrence of UV induced DNA damage in conjunctival melanoma based on a genome-wide sequencing of the two tumour cases which further reinforces an association between solar radiation and tumour development.

Ocular melanoma represents a unique model in that two different types of melanoma occur with different levels of exposure to solar radiation providing an exceptional duality model for studying the effect of solar radiation on melanoma occurrence. External ocular melanoma consists of eyelid and conjunctival melanomas which are generally directly exposed

**Table 3** Comparison of cutaneous, uveal and conjunctival melanoma characteristics

| Origin | Conjunctival melanoma<br>Melanocytes located in the basal layer of the conjunctiva | Uveal melanoma<br>Melanocytes situated in the stroma of the uveal layer of the eye | Cutaneous melnoma<br>Melanocytes located in the basal layer of the epidermis of the skin |
|---|---|---|---|
| Rate per million | 0.4 | 4.9 | 153.5 |
| Male vs. female rate per million | 0.4 vs.0.4 | 5.7. vs. 4.4 | 139.7 vs. 125.2 |
| Trends in incidence | Rising | Stable | Rising |
| Role of UV light as risk factor | Probable | Still uncertain | Well supported |
| Mean age | 57.4 | 58 | 55.3 |
| White: black ratio | 2.6:1 | 8–10:1 | 16:1 |
| Metastasizing | Lymphogenous and hematogenous | Hematogenous | Lymphogenous and hematogenous |
| Most common sites of metastases | Lymph nodes (cervical, preauricular, parotid and submandibular)<br>Lungs, liver, skin, brain, adrenals | Liver (93%)<br>Lung (24%)<br>Bones (16%)<br>Rare metastases to regional lymph node | Skin (13–38%)<br>Distant lymph nodes (5–34%)<br>Distant subcutaneous tissues (32%)<br>Lung (18–36%)<br>Liver (14–20%)<br>CNS (2–20%)<br>Bone (4%–17%) |
| Five year Survival | 86.3% | 81.6% | 80.8% |
| Treatment | Mostly surgical excision with adjuvant therapy (cryotherapy, brachytherapy, topical chemotherapy) | 62.5 radiotherapy<br>28.3% surgery | 91.5% surgery |
| Targeted therapy | No | Anti-BRAF, anti MEK (testing in progress) | Anti-BRAF, anti MEK |
| Immunotherapy | Experimental application | Anti-CTLA4, anti-PD1, anti-PDL1 (testing in progress) | Anti-CTLA4, anti-PD1, anti-PDL1 |

UV: ultraviolet; CNS: central nervous system; BRAF: BRCA-1 associated protein; MEK: CTLA4: cytotoxic T-lymphocyte-associated protein 4; PD:1 programmed cell death 1; PDL1: programmed cell death ligand 1

to the sunlight. Conversely, internal (uveal) melanoma has very limited exposure to solar radiation [57].

It is hypothesized that sunlight may have dual effects on malignant tumors: a direct mutagenic effect on tissue exposed to the sunlight and potential protective effect mediated through the activities of vitamin D on tissues which are not exposed [57]. Activated vitamin D inhibits the growth, stimulates differentiation and induces apoptosis of various malignant tumors in vitro and inhibits the growth invasion and metastasis of malignant tumors in experimental animal models [57]. Epidemiological studies indicate that deficiency and low serum levels of vitamin D are associated with the increased incidence of various malignant tumors as well as a higher mortality rate [57]. A study conducted on melanoma cells in vitro demonstrates the value of vitamin D as an inhibitor of the growth of human malignant tumors [57].

There is also some evidence supporting the influence of genetic susceptibility or other host factors on melanoma development [16, 59]. Thus these differences may be due to genetic alterations during the development of the melanocytes on distinct locations. In fact the DNA alterations observed in melanomas from anatomical sites with different levels of UV radiation exposure were shown to be body site specific [60].

## Conclusion

Conjuntival melanoma is a rare tumour however is still responsible for the death of a significant proportion of affected patients. The incidence of conjunctival melanoma is increasing with the rate being comparable to that observed in cutaneous melanoma in white populations. Ultraviolet radiation exposure contributes to this increased incidence as proposed for skin melanoma. In recent times much progress has been made in the diagnosis, treatment and prognostic outcome of conjunctival melanomas. Local tumour control has improved considerably by administering adjunctive therapy. Improvement in local treatment does not however insure an increased survival rate and new treatment options in patients with metastatic disease are necessary. Emerging knowledge of molecular changes underlying conjunctival melanoma development promises a new perspective for improved and novel targeted therapeutic agents. Given the genetic similarities between cutaneous and conjunctival melanomas, future therapies for metastatic cutaneous melanomas may possibly be applied in the treatment of the conjunctival form, particularly in respect to the use of biological therapies. This will undoubtedly lead to advancement in systemic treatment of patients with metastatic

disease as well as the prevention of spreading in those having tumours with high metastatic potential. Genetic analysis and multicenter studies will enable us to identify patients who may benefit from the use of new therapeutic agents and allow a better insight into conjunctival melanoma pathogenesis and biological behaviour.

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