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ASPECTS ON THE MANAGEMENT OF SALIVARY GLAND MUCOEPIDERMOID CARCINOMA IN FINLAND

Katri Aro

ACADEMIC DISSERTATION

To be presented, with the permission of the Medical Faculty of the University of Helsinki, for public examination in the Auditorium of the Department of Otorhinolaryngology – Head and Neck Surgery, Haartmaninkatu 4E, Helsinki, on the 14th of December 2012 at 12 noon.

Helsinki 2012

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ISBN 978-952-10-8413-3 (paperback)
ISBN 978-952-10-8414-0 (PDF)
http://ethesis.helsinki.fi
Unigrafia Oy, Helsinki 2012

To Aapo, Matias, and Aamos

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LIST OF ORIGINAL PUBLICATIONS

This study is based on the following publications indicated in the text by their roman numerals.

I Aro K, Leivo I, Mäkitie AA. Management and outcome of patients with mucoepidermoid carcinoma of major salivary gland origin: a single institution's 30-year experience. Laryngoscope. 2008 Feb; 118(2): 258-262.

II Aro K, Rosa LE, Bello IO, Soini Y, Mäkitie AA, Salo T, Leivo I. Expression pattern of claudins 1 and 3 - an auxiliary tool in predicting behavior of mucoepidermoid carcinoma of salivary gland origin. Virchows Arch. 2011 Mar; 458(3): 341-348.

III Aro K, Leivo I, Grénman R, Mäkitie AA. Paediatric salivary gland cancer in Finland. Int J Pediatr Otorhinolaryngol. 2012 Sep; 76(9): 1304-1307.

IV Aro K, Klockars T, Leivo I, Mäkitie AA. Familial predisposition for salivary gland cancer in Finland. Submitted 2012.

ABBREVIATIONS

ACC	Acinic cell carcinoma
AdCC	Adenoid cystic carcinoma
AFIP	Armed Forces Institute of Pathology
CRT	Chemoradiotherapy
cN0	No clinically evident nodal metastases
cN+	Clinically evident nodal metastases
CT	Computed tomography
DFS	Disease-free survival
FNAB	Fine needle aspiration biopsy
FNAC	Fine needle aspiration cytology
FS	Frozen section
HG	High grade
IMG	Intermediate grade
LG	Low grade
MEC	Mucoepidermoid carcinoma
MRI	Magnetic resonance imaging
ND	Neck dissection
NPC	Nasopharyngeal carcinoma
OS	Overall survival
pN+	Pathologically positive nodal metastases
RT	Radiotherapy
SCC	Squamous cell carcinoma
SGC	Salivary gland cancer/carcinoma
US	Ultrasonography

ABSTRACT

According to the Finnish Cancer Registry, salivary gland cancer (SGC) is rare, with approximately 50 to 60 new cases in Finland annually. For pediatric patients, the Finnish Cancer Registry reported 15 new cases between 1990 and 2009. The wide spectrum of different subtypes makes this group of diseases difficult to diagnose, grade, and treat. Due to the rarity of these diseases, no specific risk factors are known, and long-term outcome data are difficult to obtain. In adults, 20% of parotid gland tumors, 50% of submandibular gland tumors, and 90% of sublingual gland tumors are malignant. Most tumors of the minor salivary glands are malignant. However, in the pediatric patient population, 50% of parotid gland tumors are malignant, but only 10% of submandibular gland tumors do.

Mucoepidermoid carcinoma (MEC) is the most common (29 to 35%) malignant neoplasm of the major and minor salivary glands in both adults and children. According to the WHO, MEC is classified into three grades: low grade (LG), intermediate grade (IMG), and high grade (HG). These three grades have different growth potentials as well as different potentials to metastasize and to recur; consequently, they show major differences in outcome. Correct histopathological diagnosis thus remains vital.

We evaluated the prevalence, treatment, and outcome of 52 Finnish patients with MEC of the major salivary glands. All LG-MECs (23/52) were localized to the primary site, and most patients with LG-MEC were treated and cured surgically. The three-year overall survival rate was 95% for LG-MEC, 67% for IMG-MEC, and 55% for HG-MEC patients. Patients with LG-MEC experienced no recurrences during the three-year follow up. IMG-MEC (7/52) more closely resembled HG-MEC (20/52) in its biological behavior, contradicting reports from other institutions. Patients with IMG-MEC had cervical nodal metastases in 43% of cases, and 67% developed locoregional or distant metastases during follow up. Radiotherapy was administered for 86% of IMG-MEC patients. In the HG-MEC group, nodal metastases were present at presentation in 50% of patients, and 80% also received radiotherapy. Our results suggest an aggressive treatment protocol for IMG-MEC, and that surgery seems adequate for LG-MEC.

Claudins are integral protein components of tight junctions contributing to tight sealing between cells. Deregulation of claudins leads to loss of cell integrity, uncontrolled cell proliferation, advantageous potential for tumor growth and metastatic potential. No previous studies have explored the possible role of claudins in SGC. Immunohistochemical studies on claudins 1, 3, 4, and 7 for 39 major salivary gland MEC patients have revealed statistically significant differences in staining intensities. The high intensity for claudin-1 increased the likelihood of LG tumors with a favorable prognosis. HG- and IMG-MEC tumors more often had higher intensities for claudin-3, portending a worse prognosis. This feature again emphasizes that IMG-MEC differs from LG-MEC. Claudins may, therefore, aid in grading tumors correctly and also serve as surrogate prognostic protein markers. The extent of immunostaining had less impact on grading than anticipated. The intensity of claudin-1 immunostaining classified tumors correctly in 90% of cases corresponding to conventional histopathological features.

Between 1992 and 2011, 10 new pediatric SGC cases were identified. No gender predominated, and the median age was 14 years. The majority of SGC cases were parotid gland tumors (7/10); all tumors were LG and localized to the primary site. No recurrences occurred during the 7-month to 14-year follow up. MEC was the most common histological subtype (50%), which reflects results from other institutions. Because pediatric SGC is infrequent, experience with and guidelines for managing pediatric cases must be adapted from guidelines for adults with SGC. Establishing national or international cancer databases could offer help in managing these rare diseases.

Between 1974 and 2009, information was retrieved on 437 sequential SGC patients treated at the Helsinki University Central Hospital. Due to the long time period and the most often elderly patients, many were deceased at the time of the study. Consequently, the study cohort consisted of 161 patients who were alive and able to reach. Of these, 88 (55%) answered a patient questionnaire to identify possible hereditary forms of SGC as well as elevated risk for other malignancies among the relatives of SGC patients. The enquiry revealed no cases of SGC in first-degree relatives. Thus, most likely no significant familial predisposition for SGC exists in Finland.

SUMMARY IN FINNISH

Sylkirauhassyöpä on harvinainen ja Suomen Syöpärekisterin mukaan Suomessa diagnosoidaan vuosittain noin 50-60 uutta tapausta. Lapsilla sylkirauhassyöpädiagnooseja tehtiin ajanjaksolla 1990-2009 15. Sylkirauhassyövän alatyyppejä on WHO-luokituksen mukaan 24. Taudin vaihtelevien histopatologisten ominaisuuksien vuoksi diagnostiikka, kasvainten luokittelu ja hoito on vaikeaa. Tämän tautiryhmän harvinaisuuden vuoksi selkeitä riskitekijöitä ei tunneta ja pitkän aikavälin seurantatietoja on vaikea kerätä. Aikuisten korvasylkirauhasten kasvaimista 20%, leuanalussylkirauhasten kasvaimista 50% ja kielenalussylkirauhasten kasvaimista 90% on maligneja. Pienten sylkirauhasten kasvaimet ovat lähes aina maligneja. Lasten korvasylkirauhasten kasvaimista puolet on maligneja, mutta ainoastaan 10% submandibulaarirauhasten kasvaimista.

Mukoepidermoidikarsinooma (MEC) on yleisin (29-35%) aikuisten ja lasten pienten ja suurten sylkirauhasten maligni kasvain. WHO-luokituksessa MEC jaetaan kolmeen luokkaan: matalan maligniteettiasteen syöpä (LG), kohtalaisen maligniteettiasteen syöpä (IMG) ja korkean maligniteettiasteen syöpä (HG). Eri luokkien syöpäkasvaimilla on toisistaan eroava kasvutapa, metastasointi- ja uusiutumiskyky ja sen vuoksi suuret erot syövästä parantumisen suhteen. Oikea histopatologinen diagnoosi on äärimmäisen tärkeä nimenomaan tässä syöpätyypissä.

Helsingin yliopistollisessa keskussairaalassa hoidettujen 52 suurten sylkirauhasten MEC potilaiden prevalenssi, hoito ja hoitotulokset arvioitiin. Kaikki LG-MEC kasvaimet (23/52) olivat paikallisia ja suurin osa potilaista hoidettiin kuratiivisesti leikkauksella. Eri ryhmien kolmen vuoden eloonjäämisluvut olivat seuraavat: LG-MEC 95%, IMG-MEC 67%, HG-MEC 55%. LG-MEC potilailla ei havaittu yhtään paikallisresidiiviä kolmen vuoden seurannassa. Poiketen aiemmista tutkimustuloksista, IMG-MEC (7/52) muistutti biologiselta käyttäytymiseltään enemmän HG-MEC:aa (20/52). Kaulan imusolmukemetastaaseja oli 43%:lla IMG-MEC potilaista, seurannassa 67% kehitti paikallisresidiivejä tai kaukoetäpesäkkeitä. Sädehoito annettiin 86%:lle IMG-MEC potilaista. Puolella HG-MEC potilaista oli kaulametastaaseja diagnoosihetkellä ja suurin osa näistä potilaista sai liitännäissädehoidon. Näiden tulosten valossa IMG-MEC potilaiden hoidon pitää olla aggressiivista ja LG-MEC potilaiden hoidossa kirurgia on yleensä riittävä.

Claudiinit ovat proteiineja, jotka ovat solujen välisten tiiviiden liitosten olennaisia osia. Solusolu kontaktien menetys voi johtaa kasvainten kasvupotentiaaliin ja metastasointikykyyn. Claudiinien mahdollista osuutta sylkirauhassyövässä ei ole aiemmin selvitetty. Claudiinien 1, 3, 4 ja 7 immunohistokemiallisia värjäytymisominaisuuksia tutkittiin 39 suurten sylkirauhasten MEC potilaan ryhmässä. Mikäli kasvain värjäytyi voimakkaasti claudin-1:llä, oli se todennäköisesti LG ja ennuste oli hyvä. HG-MEC kasvaimet värjäytyivät voimakkaammin claudin-3:lla, jolloin ennuste oli selvästi huonompi. IMG-MEC kasvainten värjäytymisominaisuudet muistuttivat HG-MEC:aa, mikä edelleen korostaa tämän kasvaimen eroavaisuutta LG-MEC:sta. Värjäytymisen intensiteetillä oli tilastollinen merkitsevyys kasvainten luokittelun suhteen, laajuudella ei. Claudin-1 intensiteetti luokitteli kasvaimet 90% oikein verrattuna tavallisiin histopatologisiin kriteereihin. Claudiinit ovat apuvälineitä MEC:n oikeassa luokittelussa ja sitä kautta myös ennusteellisia tekijöitä.

Vuosina 1991-2011 diagnosoitiin Suomessa 10 uutta lasten sylkirauhassyöpää, joista saatiin riittävästi seurantatietoja. Suurin osa oli korvasylkirauhasen kasvaimia (7/10), kaikki olivat paikallisia, matalan maligniteettiasteen kasvaimia. Seuranta-aika vaihteli seitsemän kuukauden ja 14 vuoden välillä eikä tänä aikana tavattu yhtään taudin uusiutumista, metastasointia tai kuolemia. MEC oli yleisin histologinen alatyyppi (50%), mikä on raportoitu myös muualla maailmassa. Mediaani-ikä oli 14 vuotta eikä selkeää sukupuolieroa ollut. Lasten sylkirauhas-

kasvaimet ovat harvinaisia, minkä vuoksi hoitokokemukset on omaksuttava aikuisväestöstä. Kansalliset/kansainväliset syöpärekisterit mahdollistaisivat tämän harvinaisen tautiryhmän kattavamman hoitotulosten analysoinnin.

Helsingin yliopistollisessa keskussairaalassa hoidettiin vuosina 1974-2009 437 sylkirauhassyöpäpotilasta. Ottaen huomioon pitkän otantajakson ja sen, että sylkirauhassyöpäpotilaat ovat useimmiten iäkkäitä, useat heistä olivat tutkimushetkellä jo kuolleita. Osoitetiedot olivat saatavilla 161 potilaan osalta, ja näistä 88 (55%) vastasivat postitse lähetettyyn kyselyyn. Sen tarkoituksena oli selvittää mahdollisia perinnöllisiä sylkirauhassyöpätapauksia sekä sylkirauhassyöpäpotilaan sukulaisten mahdollista lisääntynyttä riskiä muihin syöpiin. Kyselyssä ei paljastunut yhtään ensimmäisen asteen sukulaista, jolla olisi ollut sylkirauhassyöpä. Tämän tutkimuksen valossa Suomessa ei ole osoitettavissa lisääntynyttä perinnöllistä alttiutta sylkirauhassyövälle.

1 INTRODUCTION

The salivary glands are exocrine glands that produce saliva through a series of ducts. Saliva comprises mainly water, but also contains enzymes, mucus, glycoproteins, electrolytes, and antibacterial compounds. Saliva is important for the digestion, lubrication, and swallowing of food as well as for the moistening and hygiene of the oral cavity. Paired parotid, submandibular, and sublingual glands constitute the major salivary glands. Minor salivary glands, numbering 500-1000, are present throughout the head and neck area beneath the mucosal lining. The relative proportion of mucous and serous acinar cells defines the composition of saliva. Almost all parotid gland acini are serous, most submandibular gland acini, most sublingual gland acini, and most minor salivary gland acini are mucous (Ellis et al. 1991).

A neoplasm is an abnormal mass of tissue which forms as a result of abnormal cell proliferation and can be benign, pre-malignant, or malignant. Epithelial cells which have lost their organisation, increased their cell mobility, and proliferate as mesenchymal cells are said to have undergone an epithelial-mesenchymal transition (EMT), which is characteristic of proliferating cells. In cancer, genes which regulate cell growth and differentiation must undergo transformation. Cancer cells exhibit stem cell characteristics with the potential to differentiate into various cell types and generate metastases (Gold et al. 2009). Individually, cancer has no known specific cause, as it involves multiple possible intrinsic and extrinsic causative factors (Yu et al. 2002).

Salivary gland cancer (SGC) is a rare carcinoma of the salivary gland tissue accounting for less than 0.5% of all malignancies and less than 5% of all head and neck malignancies worldwide. SGC is the sixth most common head and neck malignancy in Finland with approximately 50 to 60 new cases annually (www.cancerregistry.fi).

Parotid gland tumors account for 64 to 80%, submandibular gland tumors for 7 to 11%, sublingual gland tumors for less than 1%, and minor salivary gland tumors for 9 to 23% of salivary gland neoplasms (Eveson 2011). The risk for malignancy in adults varies according to site: parotid gland tumors are malignant in 15 to 32% of cases, submandibular gland tumors in 41 to 45%, sublingual gland tumors in 90%, and minor salivary gland tumors in 50 to 90% of cases, with an especially high frequency in the floor of the mouth (Eveson 2011, Barnes L, Eveson J.W., Reichart P., Sidransky D. 2005). In the pediatric population, the reverse is true: 50% of parotid gland tumors are malignant, and only 10% of submandibular gland tumors are malignant (Ribeiro Kde et al. 2002).

Finding any tumor with more versatility in histopathological features and biological behavior than SGC is difficult. The latest WHO classification of SGC was published in 2005 and describes 24 SGC entities with many subtypes (Barnes L, Eveson J.W., Reichart P., Sidransky D. 2005). These tumors exhibit various biological behaviors, responses to therapy, survival, and outcomes. Malignant tumors of the salivary glands are staged according to the TNM (T = tumor, N = node, M = metastasis) system (Sobin LH, Gospodarowicz M, Wittekind C 2009).

Unidirectional saliva secretion demands cell polarity which is accomplished by tight junctions (TJs) responsible for the barrier properties of cell-cell contacts and tissue homeostasis (Maria et al. 2008). TJ dysfunction leads to a loss of cell organization, which contributes to tumor growth and metastatic potential. Claudins are essential to TJ formation. Although researchers have studied the role of claudins in different malignancies their possible role in SGC remains unclear (Bello et al. 2008, Soini 2005).

SGC is managed primarily with surgery, and administration of postoperative radiotherapy (RT) is tailored individually. Chemotherapy is a potential treatment modality in high-grade tumors and, at the moment, is usually administered in palliative treatment, in metastatic disease, and when additional surgery is impossible (Laurie et al. 2006). The prognosis of SGC depends on the adequacy of treatment, the clinical stage, the tumor grade, and tumor location (Lima et al. 2005).

In both major and minor salivary glands, mucoepidermoid carcinoma (MEC), which represents up to 35% of malignant neoplasms, is the most common malignant tumor of the salivary glands in both adults and children (Sultan et al. 2011). MEC is classified into three categories: low (LG), intermediate (IMG), and high (HG) grades with different potentials to respond to treatment, to recur, and to metastasize. The cellular composition, mitotic figures, necrosis, anaplasia, growth pattern, and invasion are the elements in the point-based grading system of MEC according to the Armed Forces Institute of Pathology (AFIP) (Auclair et al. 1992, Goode et al. 1998).

The present study aimed to determine the incidence and outcome of MEC of the major salivary glands; to assess claudins as potential protein markers in grading and prognosticating MEC; to define the incidence, histological distribution, management, and outcome of pediatric patients with SGC; and to recognize possible hereditary SGC cases in Finland with a population-based study in the Hospital District of Helsinki and Uusimaa.

2 REVIEW OF THE LITERATURE

2.1 General considerations of salivary gland cancer (SGC)

2.1.1 Epidemiology of and risk factors for SGC

Of all head and neck malignancies worldwide, SGCs comprise less than 5% (Seethala 2011, Kokemueller et al. 2005, Guzzo et al. 2006, Kupferman et al. 2010, Shapiro et al. 2006). They encompass a wide range of different histological subtypes (see section 2.1.3 WHO histological classification). Approximately 50 to 60 new cases are diagnosed annually in Finland. Compared to other parts of the Western World with higher incidence (2.5 - 3 cases per 100 000 person years; www.cancer.gov), the Finnish Cancer Registry (www.cancerregistry.fi) reports that the age-adjusted incidence rate in Finland per 100 000 person-years is approximately 0.7. The incidence is similar in other Nordic countries and between men and women (Table 1). In Alaska, Canada, and Greenland (Inuits), the age-standardized incidence rate for SGC per 100 000 person years between 1969 and 1988 was 3.7 for men and 6.0 for women (Friborg et al. 2008).

Some suggest a female predominance (Eveson 2011, Thompson 2005); others, the opposite (Ghosh-Laskar et al. 2011, Koivunen et al. 2002). All agree that the incidence is highest after 50 years of age. The association between SGC and sequential breast cancer suggests a possible causal relationship and shared genetic background between these two malignancies. The risk for breast cancer after SGC was 2.5 to 8 fold higher than that of the statistical expected risk (Berg et al. 1968, In der Maur et al. 2005).

Table 1. Age-standardized incidence rate for SGC per 100 000 person years from 1980 to 2009 (http://www.ancr.nu) (Engholm et al. 2010).

Incidence	Men	Women
Finland	0.7	0.6
Sweden	0.7	0.6
Denmark	0.6	0.5
Iceland	0.6	0.5
Norway	0.6	0.5

Generally, tobacco and excessive alcohol consumption increase one's risk for cancer. Individuals may be intrinsically susceptible to environmental factors or have a variable ability to detoxify carcinogens with variable outcomes and risk levels (Yu et al. 2002, Mork et al. 1999). Tobacco is not a known etiological risk factor for SGC, but prior RT is (Spitz et al. 1990, Boukheris et al. 2012).

Different viral infections raise the likelihood of tumorigenesis. Epstein-Barr virus (EBV) is a known risk factor for nasopharyngeal carcinoma (NPC) and Burkitt's lymphoma, and human papillomaviruses (HPV) and herpes simplex virus (HSV) may associate with oral and pharyngeal cancers (Atula et al. 1998, Venkateswaran et al. 2000). Lymphoepithelial carcinoma (LEC) accounts for 0.4% of SGC, and shares identical histological characteristics with NPC. In endemic areas, EBV is highly associated with LEC; among Inuits, it is the most common SGC subtype (Gupta et al. 2012). Researchers have suggested an unknown viral etiology since SGC is also among the most frequent malignancies occurring under immunosuppres-

sion; a 10-times increase is observed compared to immunocompetent patients (Vajdic et al. 2006).

2.1.2 Clinical manifestation and diagnostics of SGC

The clinical appearance of SGC varies from an asymptomatic, slow-growing mass to a rapidly growing, ulcerating, and painful tumor with neurological deficits (Guzzo et al. 2010). The tumor may also be confined to an intraparotid lymph node (Gupta et al. 2012). Pain is a fairly common symptom (reported by approximately 50% of patients) (Koivunen et al. 2002). Rarely, parotid gland tumors may extend to the base of the skull and cause cranial nerve symptoms. Tumors of the oral cavity and sinuses can cause facial pain and nasal obstruction.

The assessment of a salivary gland tumor consists of a thorough clinical history, observation and palpation, different imaging techniques, fine needle aspiration cytology (FNAC), frozen section (FS) analysis, and conventional histopathology.

US (combined with fine needle aspiration biopsy (FNAB)) is usually the first radiological investigation due to its cost-effectiveness and accessibility (Eveson 2011, Guzzo et al. 2010). Larger scale evaluation of the tumor size and extent into surrounding tissues (in cases of tumors of the deep lobe of the parotid gland and in the evaluation of minor salivary gland tumors) warrants CT and MRI imaging modalities (Shah 2004). Positron emission tomography combined with CT (PET-CT) may aid in defining tumor extent and the need for additional surgery with a sensitivity of 74%, and specificity of 100% (Adelstein et al. 2011, Razfar et al. 2010). PET-CT helps in detecting distant metastatic disease. Razfar and colleagues (Razfar et al. 2010) showed that PET-CT helped and guided the management of 47% of patients with SGC. No significant difference was observed between different SGC grades.

Open biopsies should be avoided in salivary gland tumors due to possible nerve damage or tumor cell seeding from the region of the biopsy (Eveson 2011).

In salivary gland lesions, FNAC sensitivity is 28 to 55%, specificity 92%, and the rate of false negatives 38% (Koivunen et al. 2002, Atula et al. 1996). The evaluation of FNAC depends on the pathologist's experience and on the FNAB technique (David et al. 2007). FNAB is fast, easy, cheap, and a practical method for assessing the need for further treatment modalities (Christensen et al. 2010). If the specimen is malignant, the distinction between a primary tumor and a metastasis is vital (David et al. 2007). Christensen and colleagues (Christensen et al. 2010) analyzed cytological and histological diagnoses in salivary gland lesions with a high overall accuracy of 93%, sensitivity for malignancy of 83%, and specificity of 99%. FS of salivary gland malignancies lacks reliability in LG tumors, but may prove useful perioperatively (Eveson 2011, Gnepp et al. 1987). And the higher the malignancy, the higher the sensitivity of FS analysis.

2.1.3 WHO histological classification (2005)

Malignant epithelial salivary gland tumors (Barnes L, Eveson JW, Reichart P, Sidransky D 2005).

Acinic cell carcinoma
Mucoepidermoid carcinoma
Adenoid cystic carcinoma
Polymorphous low-grade adenocarcinoma
Epithelial-myoepithelial carcinoma
Clear cell carcinoma, NOS
Basal cell adenocarcinoma
Sebaceous carcinoma
Sebaceous lymphadenocarcinoma
Cystadenocarcinoma
Low-grade cribriform cystadenocarcinoma
Mucinous adenocarcinoma
Oncocytic carcinoma
Salivary duct carcinoma
Adenocarcinoma, NOS
Myoepithelial carcinoma
Carcinoma ex pleomorphic adenoma
Carcinosarcoma
Metastasizing pleomorphic adenoma
Squamous cell carcinoma
Small cell carcinoma
Large cell carcinoma
Lymphoepithelial carcinoma
Sialoblastoma

2.1.4 TNM classification (2009)

Malignant salivary gland tumors (Sobin LH, Gospodarowicz M, Wittekind C 2009).

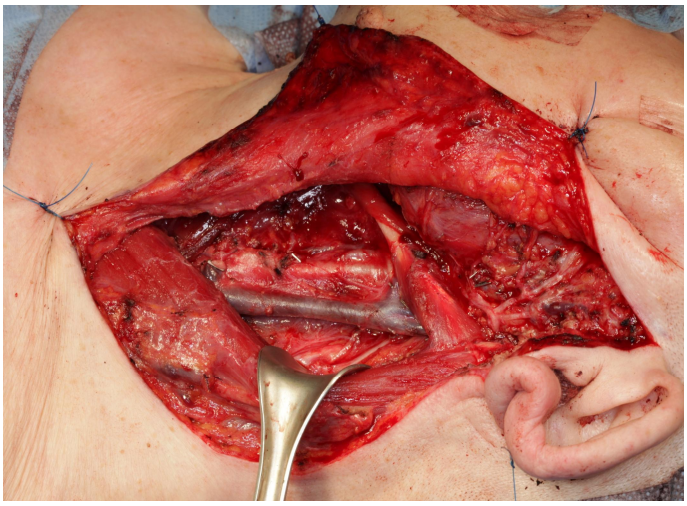
Tx Primary tumor can not be assessed **T0** No evidence of primary tumor **T1** Tumor ≤ 2 cm, no extraparenchymal (macroscopic) extension **T2** Tumor > 2 cm, but ≤ 4 cm, no extraparenchymal (macroscopic) extension **T3** Tumor > 4 cm, and/or extraparenchymal extension **T4a** Invasion to skin, mandible, ear canal, or facial nerve **T4b** Invasion to base of skull, pterygoid plates, or encloses carotid artery	**Nx** Cervical nodes can not be assessed **N0** No cervical nodes **N1** One ipsilateral metastasis ≤ 3 cm **N2a** One ipsilateral metastasis > 3 cm, but ≤ 6 cm **N2b** Multiple ipsilateral metastases, all ≤ 6 cm **N2c** Bilateral or contralateral metastases, all ≤ 6 cm **N3** Metastasis > 6 cm **M0** No distant metastasis **M1** Distant metastasis	**Stage I** T1N0M0 **Stage II** T2N0M0 **Stage III** T3N0M0 T1-3N1M0 **Stage IVA** T1-3N2M0 T4aN0-2M0 T4bN0-1M0 **Stage IVB** T4b, any N, M0 any T, N3, M0 **Stage IVC** any T, any N, M1

2.1.5 Treatment of SGC

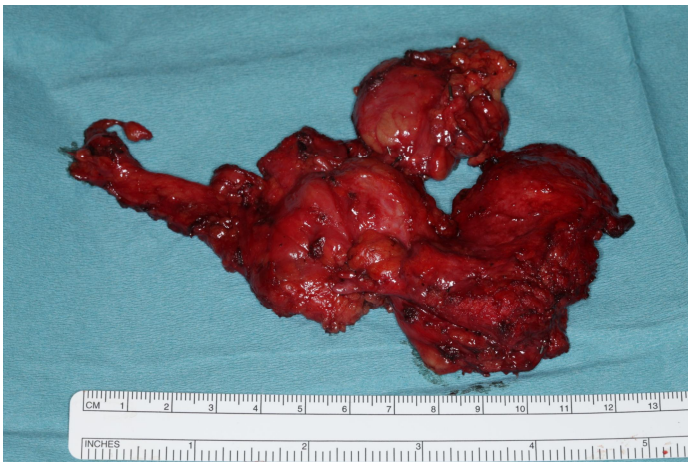
Treatment of the primary tumor

Comorbidities influence treatment selection as well as patients' responses to treatment (Terhaard et al. 2008, McHugh et al. 2012, Negri et al. 2009, Suarez et al. 2006). The treatment decision relies on past reports of treatment and outcomes for patients with similar stages of disease; surgery is often the primary treatment modality. Resection of the tumor volume is possible in a single-stage operation, accompanied by, if necessary, ND. Tumor site affects surgery. Submandibular gland resection tends to be limited to the gland capsule to limit possible damage to the marginal mandibular nerve. In the case of tumors in the retromolar area and maxilla, abundant margins are difficult to achieve without influencing the function of the specific area. The plane of the facial nerve demarcates the superficial and deep lobe of the parotid gland. Superficial parotidectomy includes resection of the gland superficial to the facial nerve. Only the glandular portion surrounding a tumor is removed in a partial superficial parotidectomy. In a total parotidectomy, all glandular tissue is resected. Identification of the facial nerve prevents inadvertent damage to it, but the risk for postoperative functional insufficiency is grater following more extensive surgery. In a radical parotidectomy (performed in more invasive tumors), total parotidectomy is extended to include excision of the facial nerve.

Figures 1 and 2 illustrate the operating field after total parotidectomy and ND.



Figures 1 and 2.
A 77-year-old female patient with parotid gland cancer after total parotidectomy, ND levels I B, II-IV, V A (spared vena jugularis interna, musculus sternocleido-mastoideus, nervus acces-sorius).



ND specimen included three metastases out of 17 lymph nodes without extracapsular spread. Courtesy of Docent Timo Atula.

Treatment of the neck

The high rate of cervical lymph node metastases in HG tumors and in advanced-stage tumors warrants elective ND (Lima et al. 2005, Klussmann et al. 2008, Regis De Brito Santos et al. 2001, Stennert et al. 2003). In about half of SGC cases that undergo surgery, the exact grade at the time of surgery remains unknown (Lima et al. 2005, Zbaren et al. 2005). Consequently, some recommend total parotidectomy in parotid gland carcinomas regardless of grade (Klussmann et al. 2008). An occult metastasis refers to a hidden metastasis beyond the recognition of clinical and radiological examination. No consensus exists on treating cN0 neck, but a risk for occult metastases that exceeds 20% usually indicates ND. Armstrong and colleagues (Armstrong et al. 1992) reported a rate of occult metastases in major SGC of 12%. In HG tumors, the risk was as high as 50%.

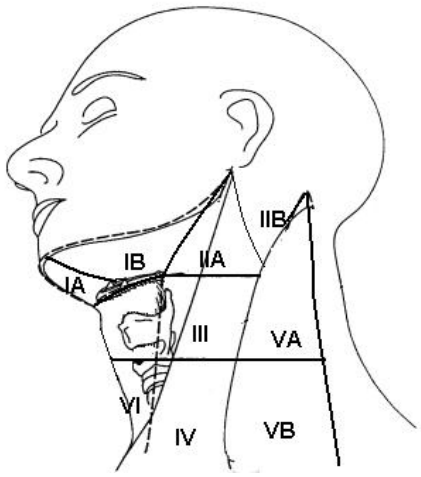


Figure 3. The levels (I-VI) of cervical lymph nodes indicated in the text by their roman numerals.

The TNM system does not differentiate intraparotid from cervical metastases. For easy and systematic description of cervical metastases, they have been divided into different levels (see Figure 3). Even though intraglandular and level II metastases are absent, occult metastases may occur at levels III and IV (Klussmann et al. 2008, Armstrong et al. 1992). If ND is limited to level II in parotid gland cancer, occult metastases are missed in 20% of cases (Armstrong et al. 1992). In a study of parotid gland cancer by Klussmann and colleagues (Klussmann et al. 2008), HG tumors included intraglandular lymph node metastases in 31% of cases, but LG tumors included metastases in 15% of cases. Patients who underwent elective ND had occult metastases - most in level II, but also in levels I and V - in 28% of cases. In therapeutic ND, lymph node metastases were present at all cervical levels. They recommend elective ND, including levels I to V, to detect all occult metastases, and total parotidectomy in all cases of parotid gland cancer to detect intraparotid lymph nodes. In their study, intraparotid lymph node metastasis was a risk factor for local failure, although it did not differentiate between nodes in the superficial and deep lobe of the parotid gland. Previously, the importance of these nodes was obscure, as they were not considered an explicit feature of worse prognosis (Healey et al. 1970).

In SGC with occult metastases, intraparotid lymph node involvement is evident in 53 to 80% of cases (Klussmann et al. 2008, Armstrong et al. 1992). Regis De Brito Santos and colleagues (Regis De Brito Santos et al. 2001) reported an incidence of occult metastases in parotid gland cancer of 37%, of which T3 or T4 tumors accounted for 77%. Stennert and colleagues (Stennert et al. 2003) reported occult metastases in 45% of primary major SGC regardless of the histological type of the tumor and T stage. Lima and colleagues (Lima et al. 2005) reported parotid gland cancer patients who underwent an elective ND to show occult cervical metastasis in 26% of cases and Zbären and colleagues (Zbaren et al. 2005) reported cN0 patients with occult metastases in 20% of cases. In their series, malignancy type was evident neither pre- nor perioperatively in 55% of cases. They recommend that ND be a routine procedure in all parotid gland primaries. Since the N stage is an independent predictor for survival, ND seems essential (Stennert et al. 2003). The occurrence of occult metastases in SGC appears in Table 2.

Table 2. Occurrence of occult metastases in patients with parotid gland carcinoma.

Study, n = number	Percent of occult metastases in patients with parotid gland carcinoma and with cN0
Armstrong 1992; n = 407 (Armstrong et al. 1992)	12
Regis De Brito Santos 2001; n = 145 (Regis De Brito Santos et al. 2001)	37
Stennert 2003; n = 160 (149/160; 93% parotid gland origin) (Stennert et al. 2003)	45
Lima 2005; n = 126 (Lima et al. 2005)	26
Zbären 2005; n = 41 (Zbaren et al. 2005)	20
Klussman 2008; n = 90 (Klussmann et al. 2008)	28
Ghosh-Laskar 2010; n = 52 (Ghosh-Laskar et al. 2011)	21

cN0 = no evidence of cervical nodal metastases at presentation

Oncological treatment

Postoperative RT is indicated in all HG tumors, in T3-4 tumors, in recurrent disease, and in tumors with positive surgical margins, perineural invasion, the presence of lymph node metastasis, and extraglandular spread (Garden et al. 1997, Terhaard 2007, Halperin,E.C., Perez C.A., Brady L.W. 2008, Armstrong et al. 1990, Mahmood et al. 2011). The target tumor volume should receive a minimum dose of 60 Gy with 2 Gy fractions (30 fractions in total) (Garden et al. 1997, Terhaard 2007, Halperin,E.C., Perez C.A., Brady L.W. 2008). T3-4 tumors, HG tumors, and in recurrent disease should receive elective RT of the neck of a dose of 50 Gy, and 60 Gy is recommended for histologically positive neck nodes (pN+) (Halperin,E.C., Perez C.A., Brady L.W. 2008). RT is administered bilaterally to the neck only when the tumor extends across the midline (Terhaard 2007). Tumors in the retromolar area and maxilla are difficult to treat with radical surgery, so patients usually need adjuvant RT; their prognosis depends on the radiosensitivity of the tumor. In the case of positive surgical margins, RT increases local control and survival rates to those of radical revision surgery (Guzzo et al. 2002).

Definitive RT has much poorer outcomes than does the treatment of combined modality; it may reach 50% five-year local control, but is an option in patients with comorbidities or if a patient refuses surgery (Terhaard 2007, Halperin,E.C., Perez C.A., Brady L.W. 2008). Palliative RT is considered in cases of distally spread disease or when treating a patient with an inoperable tumor (Terhaard 2007).

RT in the head and neck region previously had many negative consequences: pain, decreased saliva production, oral cavity infections, weakened taste, conductive hearing impairment, dental problems, and osteoradionecrosis. After the development of more sophisticated RT techniques however, these complications decreased, but their occurrence may have long-lasting implications (Schoenfeld et al. 2012).

Intensity-modulated RT (IMRT) has been among the most significant advances in the treatment of head and neck cancer, allowing surgeons to spare critical normal tissues by reducing the dose to the neighboring non-cancerous structures. The target volume of IMRT depends on the histopathology and extent of the tumor, and treatment of the neck depends on the estimated risk for cervical metastases (Terhaard 2007). Chemotherapy alone is not curative and is generally reserved for palliative treatment (Laurie et al. 2006).

The role of chemotherapeutic agents in metastatic or locally advanced SGC is of interest in cases which are unsuitable for local therapy. Paclitaxel shows modest response in recurrent or metastatic salivary gland MEC and acinic cell carcinoma (ACC), but not in adenoid cystic carcinoma (AdCC) with a median survival time of 13 months (Gilbert et al. 2006). Chemotherapy may only delay the inevitably unfavorable outcome of these tumors.

SGC patients who receive treatment of combined modality usually have a tumor of higher grade and stage (Schoenfeld et al. 2012). Schoenfeld and colleagues (Schoenfeld et al. 2012) studied chemotherapy combined with IMRT at a median dose of 66 Gy in 22 SGC patients treated primarily surgically. Only mild acute toxicity occurred. After chemoradiotherapy (CRT), four patients (18%) developed distant metastases. In their study (Schoenfeld et al. 2012), Schoenfeld and colleagues showed a three-year distant metastasis-free survival rate of 83% for all patients. In a median follow-up period of two years, all survived. Tenvetyanon and colleagues (Tenvetyanon et al. 2009) compared postoperative CRT and RT for locally advanced SGC. Their study showed a three-year overall survival (OS) to be 83% in the CRT group and 44% in the RT group. These studies show a potential role for CRT in selected HG tumors, but the selection criteria for treatment remain undefined. Due to the rarity of the disease and the variety of histological subtypes, studies of the role of chemotherapy in the treatment of advanced-stage SGC are difficult to conduct.

The ongoing RTOG 1008 trial, a randomized phase II study of adjuvant concurrent radiation and chemotherapy versus radiation alone in resected high-risk SGC, will investigate the additional value of chemotherapy in the postoperative setting (www.rtog.org/ClinicalTrials).

Because some SGCs express different hormone and molecular markers, molecular targets for therapy are possible (Adelstein et al. 2011). However, this type of approach to therapy requires clinical trials. Anti-EGFR therapy provided stability of disease progression in 50% of SGC patients with relapses or metastases (Locati et al. 2009a). Cetuximab delayed disease progression in all salivary gland AdCC, but the number of MEC (2/30; 7%) in the study was limited in order to draw conclusions on the efficacy of this treatment modality in MEC patients.

2.1.6 Prognosis of SGC

Prognosis of SGC depends on the adequacy of treatment, the clinical stage, the tumor grade, and tumor location. Lima and colleagues (Lima et al. 2005) had locoregional recurrences in 20% of parotid gland cancer cases, mainly in HG and advanced-stage tumors. Consequently, they recommend postoperative RT for these tumors. In aggressive tumors treated with surgery and adjuvant RT local failure rates reach 20% (Schoenfeld et al. 2012). The main cause of disease-related death is distant metastasis, usually in the lungs, bone, and liver (Guzzo et al. 2010). Clinical stage and facial nerve involvement may predict one's risk for distant metastasis in parotid gland cancer (Gallo et al. 1997). Distant metastases occur in 31% of HG parotid gland cancers and in 18% of LG cancers (Gallo et al. 1997). HG SGCs have a five-year survival rate of 40%, and LG and IMG SGCs have a five-year survival rate of 85% to 90% (Lima et al. 2005, Seethala 2011). Histological grade is an independent prognostic factor, but clinical stage also correlates with outcome (Seethala 2011).

2.2 Salivary gland mucoepidermoid carcinoma (MEC)

2.2.1 Definition, epidemiology, and etiology of MEC

MEC is a glandular malignant epithelial neoplasm composed mainly of mucus, intermediate, and epidermoid cells. The tumor may be variably encapsulated or infiltrative and show cysts of variable sizes.

Paul Lecène described the mucoepidermoid tumor entity, which differs from squamous and mixed tumors as early as 1908. It was previously known as a mucoepidermoid tumor that includes a benign tumor and as a tumor with metastatic potential (Stewart et al. 1945). After acknowledging its malignant potential even in the “benign” group, in 1953 the tumor acquired the more appropriate term mucoepidermoid carcinoma (Foote et al. 1953, Foote,F.W.,Jr and Frazell,E.L. 1954).

MEC accounts for 12% of all salivary gland tumors (Ellis et al. 1991). Of minor and major salivary gland malignancies, MEC constitutes approximately 30% (McHugh et al. 2012, Guzzo et al. 2002, Luna 2006). Past reports indicate that, for unknown reasons, British figures for MEC (2%) are much lower (Eveson et al. 1985). MEC is the most common salivary gland malignancy in both children and adults (Sultan et al. 2011, Thompson 2005, Luna 2006, Ozawa et al. 2008, Clode et al. 1991). Some studies suggest a possible female predominance, which varies according to tumor site (Healey et al. 1970, Guzzo et al. 2002, Stewart et al. 1945, Foote et al. 1953, Luna 2006, Spiro et al. 1978, Plambeck et al. 1996).

Salivary gland tumors associate with ionizing radiation. Malignant tumors predominate in patients with a history of RT, in which cases the most common subgroup is MEC (Luna 2006, Whatley et al. 2006, Reulen et al. 2011). After the atomic bombings in Japan in 1945, the most frequent salivary gland tumor, with an incidence of 44%, was MEC (Saku et al. 1997). After the nuclear accident in Chernobyl in 1986, no clear incidence peaks of SGC occurred in Finland (Finnish Cancer Registry). Evidence indicates that MEC occurs after RT for leukemia, Langerhans histiocytosis, and NPC (Whatley et al. 2006, Hicks et al. 2000). Patients with a history of RT to the head and neck area or chemotherapy are at higher risk for SGC. Kaste and colleagues (Kaste et al. 1994) reported eight parotid gland carcinomas, seven of which were MEC, after initial cancer treatment, which included irradiation to the head and neck area. Their study showed that of all cancers diagnosed during a certain period, SGC accounted for 6%. Of all primary cancers during the same period, SGC accounted for 0.08%. The latency period after initial cancer treatment before SGC diagnosis ranged from 4 to 14 years. Patients who have received treatment for a malignancy in childhood are at 10 to 12% higher risk for a second malignancy in 20 years (Whatley et al. 2006, Armstrong et al. 2011).

2.2.2 Histopathology of MEC

Three cell types usually comprise MEC: mucous cells producing mucus, epidermoid cells with squamoid differentiation, and intermediate cells that may predominate in numbers (Ellis et al. 1991, Goode et al. 1998, Godballe et al. 2003). Columnar, cuboidal, and clear cells are often components of MEC (Evans 1984). Intermediate cells are important for MEC identification because they show a gradual transition from small basal cells to other cell components. The term progenitor or maternal cell seems appropriate to describe this feature of the cell (Ellis et al. 1991). The intermediate cell has the ability to differentiate into an epidermoid, mucous or clear cell. The proportion of these cells varies between different MEC grades. The common finding in all grades, a feature that characterizes MEC, is the presence of intracellular mucin, the recognition of which sometimes requires additional sections and staining for

mucins (Stewart et al. 1945). It was evident as early as the 1940s that some cases required additional staining to differentiate poorly differentiated MEC from squamous cell carcinoma (SCC) (Stewart et al. 1945). In some cases, if anaplasia was present, MEC was classified as SCC, even in the presence of mucin (Gray et al. 1963).

MEC is positive for cytokeratin and expresses variable staining for epithelial membrane antigen (EMA), carcinoembryonic antigen (CEA), and S-100 (which belongs to the family of calcium binding proteins), but myoepithelial markers (actin, calponin) are negative (Fletcher 2007).

2.2.3 Variants and differential diagnosis of MEC

MEC varies depending on the proportion of the predominating tumor cell. Its variants include clear cell MEC, sclerosing MEC with or without eosinophilia, unicystic MEC, sebaceous MEC, psammatous MEC, spindle cell MEC, goblet cell aggressive MEC, and oncocytic MEC. The p63 protein expressed in myoepithelial and basal cells, is a marker of myoepithelial differentiation and is overexpressed in some tumor types. Oncocytic MEC is diffusively positive for p63, an immunohistochemical finding which could help in the differential diagnosis of MEC (Weinreb et al. 2009). HG-MEC showed an increased staining for p63 (Seethala et al. 2005).

The differential diagnosis of MEC includes various tumors: necrotizing sialometaplasia, inverted papilloma, SCC, adenosquamous carcinoma, cystadenoma, cystadenocarcinoma, sebaceous carcinoma, ACC, hyalinising clear cell carcinoma, clear cell oncocytoma, and metastatic renal cell carcinoma.

2.2.4 Prognostication of MEC

Grading

In MEC, tumor grading is essential since the biological behavior of tumors varies considerably between grades (Seethala 2011). Grading criteria and the number of grades are controversial, however (Evans 1984). The necessity for a gradually increasing grading system became evident because many tumors share histopathological characteristics of aggressiveness (Foote et al. 1953, Foote,F.W.,Jr and Frazell,E.L. 1954). Healey and colleagues (Healey et al. 1970) agreed to include in histopathology medium-grade tumors which more closely resemble LG tumors. Spiro and colleagues (Spiro et al. 1978) applied more grading criteria and added staging. MEC is currently divided into LG, IMG, and HG based on morphology and cytology. In general terms, mucous cells predominate in LG-MEC, intermediate cells predominate in IMG-MEC, and epidermoid cells predominate in HG-MEC.

Pathologists have accepted the three-tiered grading system for the histological evaluation of MEC, although none of the three best-known grading systems is clearly superior to the others or sees consistent and universal use. Luna (Luna 2006) reviewed and compared the grading systems designed by Batsakis and Luna, who modified Healey's grading system; Brandwein's system; and the AFIP system (Table 3) (Auclair et al. 1992, Goode et al. 1998, Healey et al. 1970, Batsakis et al. 1990, Brandwein et al. 2001). He concluded that the first two systems were more alike than the AFIP system. Consequently, a standardized, easy to use, reproducible, and accurate grading system is necessary. Brandwein and colleagues (Brandwein et al. 2001) stated that the criteria proposed by the AFIP system downgraded MEC. Luna (Luna 2006) agreed that the Brandwein system was objective and easy to repro-

duce. It is noteworthy that in the absence of a grading system in universal use, studies of MEC are inconsistently comparable.

LG-MEC is non-invasive, has more mucous cells and fewer solid cellular areas, and shows no keratinisation. IMG-MEC shows invasive borders with predominantly intermediate cells with fewer and smaller cysts. It is a distinctive tumor entity rather than a mixture of the other two grades. Solid cellular proliferations characterize infiltrative HG-MEC with evident atypia, anaplasia, mitoses, and necrosis. The presence of intermediate and mucous cells and the production of intracellular mucin differentiate these tumors from SCC, even though HG-MEC may show only little mucin production (Luna 2006, Evans 1984, Nance et al. 2008).

Problems in the histological classification of MEC cause discrepancies in grading. Since the establishment of a description for MEC, many other histopathological diagnoses of SGC have emerged: salivary duct carcinoma (1968), adenosquamous carcinoma (1968), epithelial-myoepithelial carcinoma (1972), polymorphous low-grade adenocarcinoma (1983), basal cell adenocarcinoma (1990), mammary analogue secretory carcinoma (MASC; 2010), and cribriform adenocarcinoma of minor salivary gland origin (CATS; 2011) (Chenevert et al. 2011, Skalova et al. 2011, Skalova et al. 2010). These new tumor descriptions and modern diagnostic criteria make it easier to achieve histologically a more precise and accurate diagnosis.

Table 3. Different grading systems for MEC.

	AFIP 1996, 2008 (point based) (Auclair et al. 1992, Goode et al. 1998) **LG = 0-4 points** **IMG = 5-6 points** **HG = ≥ 7 points**	**Brandwein 2001** (point based) (Brandwein et al. 2001) **LG = 0 points** **IMG = 2-3 points** **HG = ≥ 4 points**
Intracystic space	< 20%; +2	< 25%; +2
Neural invasion	+2	+3
Necrosis	+3	+3
Mitotic activity	≥ 4 per 10HPF; +3	> 4 per 10HPF; +3
Anaplasia	present; +4	pronounced nuclear atypia; +2
Pattern of infiltration		in small nests and islands; +2
Vascular or lymphatic invasion		+3
Bone invasion		+3

HPF = high power fields

Batsakis and Luna 1990 (Modified Healey, qualitative) (Batsakis et al. 1990).

LG	**IMG**	**HG**
Cystic cells, differentiated mucin-producing epidermoid cells, minimal pleomorphism, rare mitoses, pools of extravasated mucin	Solid nests of cells, moderate pleomorphism, few mitoses, prominent nuclei and nucleoli, invasive quality, chronic peripheral inflammation	Predominantly solid, but may also be almost totally glandular, poor differentiation of cells, considerable pleomorphism, frequent mitoses, invasion, inflammation less prominent

Cytokeratins and mucins

Distinguishing HG-MEC from SCC is difficult. Sobral and colleagues (Sobral et al. 2002) showed that these tumors have different immunohistochemical staining for cytokeratins. Their study suggests that SCC draws attention with highly positive staining for cytokeratin 14; in HG-MEC, staining is only focal. Their study also states that areas of squamous metaplasia, present in both tumor types, stain differently for cytokeratins 10 and 13. In MEC, well-developed mucous cells seldom react to cytokeratin.

Mucins are glycoproteins produced by epithelial tissues. Compared to normal salivary gland tissue, tumors show altered mucin expression. Their expression in cancer cells varies in quantity and form and according to tumor type and site. Many adenocarcinomas exhibit atypical expression of mucins, and MUC1 is commonly overexpressed in these cancers, thus correlating with worse prognosis (Handra-Luca et al. 2005). Handra-Luca and colleagues (Handra-Luca et al. 2005) studied mucin expression in MEC. They found that intermediate cells express cytoplasmic MUC2 and MUC5AC, but might show no mucin in histochemical stains. Lack of MUC1 aids in determining more indolent tumors, but this does not correlate with grade. Their study also demonstrated that the expression of MUC4 is more frequent in LG-MEC than in HG-MEC. The staining characteristics of MEC for mucin and cytokeratin are not specific enough to differentiate MEC from other adenocarcinomas. Head and neck adenosquamous carcinomas and necrotizing sialometaplasia show MUC5AC, thus indicating the presence of these mucins in benign lesions as well as in non-MEC tumors (Handra-Luca et al. 2005). The staining characteristics for MUC5AC may help to distinguish HG-MEC from SCC (Handra-Luca et al. 2005).

Proliferation markers

In addition to histopathological tumor features, immunohistochemical markers of cell proliferation aid in evaluating aggressiveness and prognosis. PCNA (proliferating cell nuclear antigen) assesses cell proliferation and is therefore a tool for grading MEC; higher-grade tumors show a higher percentage of PCNA-positive cells (Luna 2006, Cardoso et al. 2000). Five-year disease-free survival (DFS) and five-year OS, however, lack statistical difference between low and high expression of PCNA in MEC, although it is evident in AdCC (Stenner et al. 2012).

Ki-67 protein is a cellular proliferation marker present in all phases of the active cell cycle, but absent from the resting cells. Studies have investigated Ki-67 immunoreactivity, and its value as a prognostic marker. The MIB-1 is a monoclonal antibody that detects Ki-67 and functions in formalin-fixed paraffin-embedded sections. Immunohistochemical staining for Ki-67 correlates with aggressive tumor behavior when the proliferation index (MIB-1 index) is > 10%, but a prognostic view suggests that its significance remains equivocal (Seethala 2011, Luna 2006, Skalova et al. 1994, do Prado et al. 2011). Kiyoshima and colleagues (Kiyoshima et al. 2001) reported the Ki-67 immunostaining ratio to correlate to both the grade and clinical behavior of MEC. An MIB-1 index > 15% correlates with worse survival among T1 and T2 tumors as well as among tumors without cervical nodal metastasis (Vacchi-Suzzi et al. 2010). In a study by Luukkaa and colleagues (Luukkaa et al. 2006), the MIB-1 index correlated significantly with prognosis in MEC patients. The researchers chose a value of 20 as a cut-off point; tumors exceeding this value ended up with a worse five-year OS, which was statistically significant. Thus far, Ki-67 immunoreactivity is absent from MEC grading schemes.

Other molecular markers

p53 gene alteration is among the most vital steps in carcinogenesis, but is less evident in tumorigenesis of MEC. The protein product of human papillomavirus degrades tumor suppressor p53. Mutations of p53, HPV status, and their possible synergism in prognosis remain unclear (Gold et al. 2009). Immunoexpression of p53 and its correlation with the histological grade of MEC and prognosis remains unsettled (Seethala 2011, Luukkaa et al. 2006).

In various adenocarcinomas (of the pancreas and ovary), elevated levels of tumor markers show prognostic significance. Carbohydrate antigens CA19-9 and CA 125 immunostaining characteristics provide no auxiliary tool for assessing of MEC tumor behavior (Okamura et al. 2002).

Overexpression of epidermal growth factor receptor (EGFR) is evident in most head and neck SCC (Pivot et al. 2005). Its activation induces cell proliferation and blocks apoptosis, and is involved in invasion of the tumor; it therefore associates with poor prognosis (Gold et al. 2009, Pivot et al. 2005). Anti-cancer therapeutics aimed at EGFR emerged after the identification of this oncogene (Prenen et al. 2008).

Claudins and tight junctions

In salivary glands, cell polarity is crucial for unidirectional saliva secretion (Maria et al. 2008). Tight junctions (TJs) contribute to ion flux and tight sealing between epithelial and endothelial cells (Soini 2005, Morin 2005). By doing so, they maintain tissue homeostasis and cell polarity (Maria et al. 2008, Morin 2005, Tsukita et al. 2008, Peppi et al. 2004, Michikawa et al. 2008, Tzelepi et al. 2008). TJs may also regulate proliferation and differentiation, among other cellular functions, due to their capacity to recruit signalling proteins (Maria et al. 2008, Peppi et al. 2004). Occludin, claudins, and junctional adhesion molecules are proteins comprising TJs (Morin 2005). Other proteins, such as connexins, integrins, and E-cadherin, are also vital for the development of apical-basal polarity (Tsukita et al. 2008). Tumor cells that display decreased differentiation and loss of cell-to-cell adhesion often lack advanced cell organization (Morin 2005). Loss of cell integrity, decreased polarity, and differentiation lead to increased influx of nutrients and growth factors, thereby contributing to advantageous tumor growth and metastatic potential (Bello et al. 2008, Tsukita et al. 2008, Michikawa et al. 2008, Singh et al. 2010, Miyamoto et al. 2008, Dos Reis et al. 2008, Al Moustafa et al. 2002).

The role of TJs in carcinogenesis has generated interest, as over 90% of cancers are epithelial in origin (Tsukita et al. 2008). Repeated growth stimulation of cells in pre-malignant lesions that exhibit TJ dysfunction increases the risk of carcinoma (Singh et al. 2010). Phosphorylation of claudins may be evident in decreased function of TJs (Tsukita et al. 2008).

The claudin family of proteins consisting over 20 known transmembrane proteins are the most important structural elements in TJs (Singh et al. 2010). The lack of other constituent proteins does not necessarily lead to the breakdown of TJ strands (Tsukita et al. 2008). Claudins outline the barrier function of TJs between the plasma membranes of two neighboring cells. Claudins are evident in endothelial and epithelial cells, thus encompassing tissue specificity (Soini 2005, Singh et al. 2010). They show various patterns of expression along different parts of the ductal system of the salivary gland, and different human tissues show multiple claudins (Soini 2005, Peppi et al. 2004).

Non-syndromic recessive deafness and hereditary hypomagnasemia show mutations of claudin genes, but mutations of claudin genes which cause tumorigenesis are thus far absent but possible. Their role in tumorigenesis is tissue specific and their expression can increase

or decrease (Tsukita et al. 2008, Singh et al. 2010). The intensity and extent of claudins varies in different cancers (Tsukita et al. 2008). Table 4 illustrates the expression of claudins studied in this thesis in various cancers.

Table 4. Expression of claudins studied in this thesis. Gene locus and expression pattern in normal and malignant tissues.

	Gene locus	Expression in normal salivary gland tissue	Elevated expression in malignant tumors	Diminished expression in malignant tumors
CLDN 1	3q28	Acinar and ductal cells	Lung SCC, liver, meningioma, prostate, tongue SCC	Breast, colorectal, lung adenocarcinoma, metastatic melanoma
CLDN 3	7q11.23	Acinar and ductal cells	Breast, prostate, ovarian	Bladder
CLDN 4	7q11.23	Acinar and ductal cells	Breast, prostate, tongue SCC, thyroid	Liver, meningioma
CLDN 7	17	Ductal cells	Liver, prostate, tongue SCC, thyroid	Breast, head and neck, metastatic melanoma

CLDN = claudin; SCC = squamous cell carcinoma

Claudins show site- and type-specific variation. Differences in claudins occur between adenocarcinoma and SCC. SCC of the lungs and esophagus express lower claudin-3 levels than do adenocarcinomas (Moldvay et al. 2007, Takala et al. 2007). In gastric carcinoma, the diffuse type shows diminished expression of various claudins, whereas the intestinal type shows strong expression (Soini et al. 2006). Ductal and lobular carcinomas of the breast express claudins differently; invasive ductal carcinomas express more often claudins 2 and 5 than lobular carcinomas (Soini 2004). Claudin-1-positive esophageal SCC, renal and thyroid papillary carcinomas, and lung adenocarcinomas correspond to a more favorable outcome (Tzelepi et al. 2008, Miyamoto et al. 2008, Chao et al. 2009, Fritzsche et al. 2008). In contrast to the above, overexpression of claudin-1 associates with aggressive behavior in oral SCC and renal clear-cell carcinomas, although other studies do not support this conclusion (Bello et al. 2008, Dos Reis et al. 2008, Chao et al. 2009). Upregulation of claudin-1 decreases apoptosis in NPC (Lee et al. 2010), diminished expression of claudin-3 associates with poor prognosis in gastric carcinoma, elevated expression of claudin-3 and -4 results in tumor invasion in ovarian cancer, and reduced expression of claudin-7 correlates with higher grade and stage (Soini et al. 2006, Morin 2007, Agarwal et al. 2005, Sauer et al. 2005). Claudin expression is absent in lymphomas, a feature that can distinguish these malignancies from epithelial neoplasms (Soini 2005). Claudin expression varies in the same tumor and in areas of poorer differentiation (Soini 2005). The immunohistological staining intensity and quantity of claudin-7 in tongue SCC patients can benefit prognostication (Bello et al. 2008).

Clinical significance of protein kinases

Biological targets for therapy generate considerable interest; tyrosine kinase and hormonal receptors are found in MEC as follows: c-kit, 0 to 40%; EGFR, 53 to 100%; and HER2, 0 to 38% (Guzzo et al. 2010). Of the hormone receptors in SGC, estrogen and progesterone are lacking (Locati et al. 2009b). Vascular endothelial growth factor (VEGF) expression is evident in 50% of MEC (Adelstein et al. 2011).

2.2.5 Fusion oncogenes

The creation of fusion genes is a common mechanism in oncogenesis. A t(11;19)(q21;p13) translocation has been found to yield MECT1-MAML2 fusion oncogene. These fusion oncogenes are often specific to each disease, but in other diseases, the genes involved may have different gene partners. Clear cell hidradenomas (Martins et al. 2004) and MEC (up to 70%) show MECT1-MAML2 fusions; many studies claim it is absent from Warthin tumors (Martins et al. 2004, Seethala et al. 2010, Okabe et al. 2006, Moller et al. 2008, Teixeira 2006). This translocation seems to be specific to MEC, although fusion positivity also occurs in up to 50% of HG-MEC cases (Martins et al. 2004, Seethala et al. 2010, Okabe et al. 2006). Other parameters are therefore needed to distinguish different grades. The METC1-MAML2 fusion gene is more often expressed in younger patients with smaller tumors and in LG- or IMG-MEC (up to 75%), thus portending a more favorable outcome (Seethala et al. 2010, Moller et al. 2008, Fehr et al. 2009, Bhaijee et al. 2011).

In different soft tissue and bone tumors as well as leukemias, EWSR1 gene fusion is evident (Moller et al. 2008). EWSR1-POU5F1 fusion correlates with higher grades of MEC (Moller et al. 2008). Thus, these two fusion oncogenes could distinguish two distinct grades of MEC, as fusion oncogenes are often disease-specific; in other diseases, however, the genes involved may have other fusion gene partners (Teixeira 2006).

The positive fusion oncogene (MECT1-MAML2) status varies in HG-MEC, possibly because this group can also include other HG malignancies, such as poorly differentiated adenosquamous carcinoma, salivary duct carcinoma, and adenocarcinoma NOS. Even though the translocation status seems to correlate with more indolent tumors within HG-MEC, it does not ensure a positive outcome, as lethal cases occur among translocation-positive tumor patients.

HMGA2 is a transcription factor and a stem cell marker with elevated expression levels in HG-MEC (Fehr et al. 2009). Its linkage to grading makes it a possible prognostic and therapeutic factor (Fehr et al. 2009).

2.2.6 HG-MEC and squamous cell carcinoma

How can we differentiate HG-MEC from SCC? In addition to traditional histopathological features, the following characteristics help in MEC diagnostics.

1. Glandular, cystic structures in MEC to diagnose a tumor of salivary gland origin (see section 2.2.1).
2. The presence of mucin (in mucicarmine stains) is diagnostic in MEC (see section 2.2.2).
3. The degree of keratinisation which is wide-ranging in SCC (Ellis et al. 1991).
4. CK14 positivity. In HG-MEC positivity is only focal, whereas in SCC, it is thorough (see section entitled “Cytokeratins and mucins”).
5. CRTC1-MAML2 fusion oncogene status in MEC; this fusion is specific to MEC (see section 2.2.5).

2.2.7 Treatment of MEC

Surgery is the treatment of choice for MEC, and adjuvant therapy depends on grade, stage, and surgical margins (Nance et al. 2008). According to Terhaard and colleagues (Terhaard 2007), the risk estimate for MEC with cN+, regardless of grade, is 12 to 60%, depending on

tumor location and T category. Excision for LG-MEC tumors is usually sufficient, but HG-MEC warrants broad surgery and postoperative RT. The decision of RT ought to be based on histological findings and additional clinicopathological characteristics. In a study by McHugh and colleagues (McHugh et al. 2012), most IMG-MEC patients (34/54; 62%) underwent postoperative RT due to HG features; all improved in survival compared to HG-MEC.

Chemotherapy for advanced SGC is mainly palliative, and its possible effects seem to differ according to histological subtype (Laurie et al. 2006). In a report by Laurie and Licitra (Laurie et al. 2006), the responsiveness of MEC tumor to paclitaxel seems to differ from that of AdCC. Another study showed paclitaxel to have modest response in MEC and ACC tumors; partial response in 3/14 of MEC tumors, in 5/17 of ACC tumors, but no response (0/14) in AdCC tumors. (Gilbert et al. 2006). Compared to RT, postoperative cisplatin/carboplatin-based CRT in locally advanced MEC showed no benefit in survival (Tenvetyanon et al. 2009).

Table 5 shows current treatment recommendations for MEC. Treatment for IMG-MEC tumors, however, remains controversial. The levels of ND in MEC with cN0 are poorly defined. Stennert and colleagues (Stennert et al. 2003) reported a 30% occult metastasis rate, although LG- and HG-MEC were undifferentiated. The section entitled “Treatment of the neck” discusses treatment of the neck in SGC.

Table 5. Treatment recommendations for MEC of the salivary glands.

Surgery only	Radiotherapy only	Surgery and radiotherapy	Additional neck dissection	Chemotherapy
- negative margins - T1, T2 tumors - LG histology	- palliative treatment - tumor/patient is not suitable for surgery - patient refuses surgery	- positive margins - T3, T4 tumors - pN+ - HG histology - invasion into surrounding structures	- cN+ - cN0, but: 1. T3, T4 tumors 2. HG histology 3. high risk for occult metastases	- palliative treatment - metastatic disease - tumor is unsuitable for additional surgery

2.2.8 Survival and prognosis of MEC

Reports indicate the prognosis of patients with submandibular tumors to be worse than with parotid and sublingual gland tumors. The occurrence of distant metastasis is 17-20% in parotid gland tumors, 37-42% in submandibular gland tumors, and 17% in sublingual gland tumors (Mariano et al. 2011, Bradley 2001). In a study by Goode and colleagues (Goode et al. 1998), most submandibular gland MECs were LG, and most deaths caused by MEC occurred in this group. In contrast, in the parotid gland group, most deaths occurred in patients with HG-MEC. Consequently, they recommend aggressive therapy for submandibular gland MEC regardless of grade. The aggressive nature of MEC in this site is known, although not all support this tendency (McHugh et al. 2012, Spiro et al. 1978, Brandwein et al. 2001, Nance et al. 2008).

The development of distant metastasis is a key element in prognosis. Brandwein and colleagues (Brandwein et al. 2001) reported distant metastases in 6% of their patients; all events occurred in patients with HG-MEC. Death occurred in 25% of patients (11/48), most had HG-MEC, and none had LG-MEC. Distant MEC metastases portend a poor prognosis; survival ranges from 2.3 years for minor salivary gland tumors to 2.6 years for major salivary

gland tumors (Spiro et al. 1978). The patient's age is a prognosticator for survival in MEC (Luukkaa et al. 2005). Past reports indicate significantly worse survival for HG-MEC patients compared to IMG- and LG-MEC patients (McHugh et al. 2012, Nance et al. 2008). McHugh and colleagues (McHugh et al. 2012) reported that stage and evidence of perineural invasion are independent prognostic factors. Table 6 shows the outcome of salivary gland MEC in different studies according to tumor stage and grade.

Table 6. Five-year survival rates of patients with MEC by clinical stage and grade.

Clinical stage	I	II	III
Spiro 1978; 5-year OS (%) (Spiro et al. 1978)	97	83	28
Guzzo 2002; 5-year DFS (%) (Guzzo et al. 2002)	I-II; 83-89		III-IV; 21-33
Kokemueller 2005; 5-year OS (%) (Kokemueller et al. 2005)	I-II; 92 (10-year 82)		III-IV; 50 (10-year 30)
McHugh 2012; 5-year DFS (%) (McHugh et al. 2012)	I-II; 89		III-IV; 47
Grade	1	2	3
Healey 1970; 5-year DSS (%) (Healey et al. 1970)	100	90	31
Clode 1991; 5-year DSS (%) (Clode et al. 1991)	100	70	43 (at 2 years)
Kokemueller 2005; 5-year OS (%) (Kokemueller et al. 2005)	90 (10-year 82)		38 (10-year 0)
McHugh 2012; 5-year DFS (%) (McHugh et al. 2012)	88	91	43
Ghosh-Laskar 2011; 5-year DFS (%) (Ghosh-Laskar et al. 2011)	85 (10-year 85)	81 (10-year 67)	53 (10-year 35)

OS = overall survival; DFS = disease free survival; DSS = disease specific survival

2.3 Specific features of pediatric salivary gland cancer

Though rare, SGC affects children and adolescents in less than 5% of all SGC cases (Kokemueller et al. 2005, Khadaroo et al. 1998, Ryan et al. 2011). According to the Finnish Cancer Registry, 36 pediatric SGCs occurred in Finland during the period from 1953 to 2009 (Finnish Cancer Registry 2012). Incidence among children and adolescents rises after the age of 10, but never reaches the incidence rates of adults (Ribeiro Kde et al. 2002, Sultan et al. 2011, Shapiro et al. 2006, Venkateswaran et al. 2000, Ryan et al. 2011, Orvidas et al. 2000). Pediatric SGC shows no clear gender predominance and gender predisposition varies across different studies (Ribeiro Kde et al. 2002, Sultan et al. 2011, Shapiro et al. 2006, Orvidas et al. 2000, Shikhani et al. 1988).

The parotid gland is the salivary gland most often affected, and MEC is the most common histology in pediatric SGC (Ribeiro Kde et al. 2002, Sultan et al. 2011, Kupferman et al. 2010, Shapiro et al. 2006, Hicks et al. 2000, Orvidas et al. 2000, Rahbar et al. 2006, Védrine et al. 2006, Hockstein et al. 2004). Parotid gland tumors are more often malignant (50 %) in the pediatric population than in adults (13 to 25%) (Guzzo et al. 2006, Koivunen et al. 2002, Mehta et al. 2006). Submandibular gland tumors are more often malignant in adults (50%) than in children (10%) (Shapiro et al. 2006, Hockstein et al. 2004). In the pediatric SGC pop-

ulation, clinical presentation is usually a localized, well-differentiated MEC (Sultan et al. 2011).
Surgery is the treatment of choice for pediatric SGC, and it is usually the only treatment modality (Guzzo et al. 2006, Ryan et al. 2011). It consists of partial or total parotidectomy or resection of the gland with adequate surgical margins. The necessity of ND for pediatric SGC patients remains speculative, since occult metastases are rare; this procedure is usually recommended in cases of clinically evident nodal metastases (Guzzo et al. 2006). Adjuvant RT is indicated only in cases with aggressive histological features, widely spread disease, positive or inadequate surgical margins, or perineural invasion (Orvidas et al. 2000). RT may cause negative implications in children, including facial skeletal growth, other deformities, dental problems, visual impairment, osteoradionecrosis, and a risk for second cancers in the irradiated field (Kupferman et al. 2010). Chemotherapy is reserved for pediatric SGC patients in cases of palliative care, recurrences, and in situations where additional surgery or RT is impossible (Laurie et al. 2006, Ryan et al. 2011).

LG-MEC (range, 27 to 75%) and IMG-MEC (range, 19 to 58%) are the most frequent subtypes in pediatric SGC (Guzzo et al. 2006, Hicks et al. 2000, Ryan et al. 2011, Védrine et al. 2006). The recurrence rate is only approximately 10%, although for HG-MEC it is 50% (Ryan et al. 2011). If MEC recurs in patients with a primary MEC of LG or IMG, surgical intervention is adequate to maintain a positive outcome (Guzzo et al. 2006). In primary HG tumors, however, mortality rates ranged from 33 to 100% (Hicks et al. 2000, Ryan et al. 2011).

Pediatric salivary gland tumors usually present with LG histology with a favorable prognosis (Ribeiro Kde et al. 2002, Sultan et al. 2011, Kupferman et al. 2010, Shapiro et al. 2006, Orvidas et al. 2000). Pediatric patients usually have markedly better survival rates (rising above 90%) than do adults (Sultan et al. 2011, Guzzo et al. 2006, Kupferman et al. 2010, Ryan et al. 2011, Védrine et al. 2006). Sultan and colleagues (Sultan et al. 2011) report differences in survival according to age. In their series, children had a five-year OS of 95%, whereas adults had 59%. Pediatric SGC patients are at 3% risk for developing a second cancer during the 20 years after concluding the primary treatment (Védrine et al. 2006). The expected risk for a subsequent neoplasm rises to 3- to 6-fold that of the expected risk (Reulen et al. 2011). Védrine and colleagues (Védrine et al. 2006) reported 18 pediatric patients with salivary gland MEC (11 LG, 3 IMG, 2 HG, 2 unknown grade), of whom 61% (11; 9 LG, 2 IMG) had a history of cancer in childhood. Compared to patients with MEC as the primary, MEC as a second primary had no impact on survival. Boukheris and colleagues (Boukheris et al. 2012) reported that the incidence of SGC was 39-fold higher among childhood cancer survivors treated with RT. Most primary malignancies were leukemia and lymphoma. They stressed the need for long-term follow up for these patients.

2.4 Heredity of salivary gland cancer

Familial cases of salivary gland neoplasms are rare. Reports indicate that familial cases of pleomorphic adenoma, Warthin tumor, carcinoma of the submandibular gland, MALT lymphoma, and ACC exist (Delides et al. 2005, Depowski et al. 1999, Newman et al. 1981, Klausner et al. 1994, Hayter et al. 1990). However, familial predisposition is well recognized for many cancers, in addition to environmental and habitual factors. To estimate this predisposition, Hemminki and colleagues (Hemminki et al. 1997) studied the Swedish Family-Cancer database, which includes all cancers diagnosed in Sweden since 1961. They found that parental colorectal cancer increases the risk for SGC in offspring 2.4- to 3.6-fold. The risk for any cancer in offspring is 1.3- to 1.4-fold if both parents have any form of cancer. Additional studies suggest that parental SGC increases one's risk for medulloblastoma in offspring, and having a sibling with Hodgkin lymphoma or a brain tumor increases one's risk for SGC (Hemminki et al. 2008, Hemminki et al. 2000).

Several factors indicate a possible genetic predisposition for SGC. Its incidence is 4.5- to 9-fold higher among Eskimos in Greenland than among Europeans (Albeck et al. 1993). In Greenland, 33% of SGC cases occurred in familial clusters over an 11-year period (Albeck et al. 1993). In these families, first-degree relatives were at high risk for virally associated SGC and cervical cancer (Friborg et al. 2005). One report shows familial SGC in Eskimos in Greenland in five siblings in two families (Merrick et al. 1986). The high risk for SGC is unique to the Inuits, and the risk remains after migration. This implies that genetic and environmental risk factors act already in childhood (Friborg et al. 2008). Undifferentiated SGC is associated with EBV infection, which resembles NPC histopathologically.

3 AIMS OF THE STUDY

The purpose of this study was to profile the characteristics, management, and outcome of Finnish adult MEC patients and pediatric SGC patients. No previous studies have evaluated pediatric SGC or the familial predisposition for SGC in Finland.

The specific aims were to determine:

- characteristics, management, and outcome of MEC of the major salivary glands (I).
- claudins as potential prognostic and predictive markers in MEC (II).
- prevalence, histological distribution, and outcome of pediatric patients with SGC (III).
- possible hereditary SGC cases (IV).
- treatment recommendations for MEC and for pediatric patients with SGC (I, III).

4 PATIENTS AND METHODS

4.1 Patients and study design

The Department of Otorhinolaryngology – Head and Neck Surgery, Helsinki University Central Hospital is the tertiary referral center responsible for the treatment of these malignancies in Southern Finland. All patients included in Studies I, II, and IV were diagnosed and in Studies I and II also treated at the Helsinki University Central Hospital. All tumors were staged according to the TNM classification available for each time point.

4.1.1 Study I

The aim of the study was to analyze the management and outcome of adult patients with MEC of the major salivary glands.

During a 30-year period from 1976 to 2005, 60 adult patients were diagnosed with MEC of the major salivary glands. The data of the study included demographic data on the patients, clinical and histological data on the tumor, and treatment and outcome factors. The diagnostic criteria were those of the WHO classification, and grading criteria were those of AFIP. After 1991, the designation mucoepidermoid tumor indicated LG-MEC, so we included these mucoepidermoid tumors in the study. We excluded cancers of the minor salivary glands, intrabony MEC, and pediatric patients with MEC from the study population. In a previous study (Luukkaa et al. 2005), all SGCs diagnosed in Finland from 1991 to 1996 were verified and graded again. In addition, professor Ilmo Leivo graded all IMG-MECs in this study.

Since follow-up data on eight patients were lacking, the study comprised 52 patients, of whom 31 (60%) were women. The age of the study patients ranged from 25 to 84 years. The length of follow up varied from six months to nine years. DFS was calculated as the time from diagnosis to the first event, which was either tumor recurrence or death due to MEC.

4.1.2 Study II

The aim of the study was to investigate the prognostic and predictive value of different claudins in MEC.

The individuals in Study II are a subset of Study I. Of these 60 patients with MEC, 39, of whom 24 (62%) were women, had sufficient tumor material to be included in the study. Median age was 57 years. Diagnostic criteria were those of the WHO, and grading criteria were those of AFIP. We had 17 (44%) LG-MECs, 6 (15%) IMG-MECs, and 16 (41%) HG-MECs.

Immunohistochemistry (Study II)

In Study II, we performed immunohistochemistry on paraffin-embedded blocks, 5-µm-thick sections of the tumor material. The primary antibodies were polyclonal rabbit anti-claudin-1 (clone JAY.8), polyclonal rabbit anti-claudin-3 (clone Z23.JM), monoclonal mouse anti-claudin-4 (clone 3E2C1), and polyclonal rabbit anti-claudin-7 (clone ZDM.241) - all purchased from Zymed Laboratories (South San Francisco, CA, USA). After heating the sections for 10 minutes in microwave oven in 10 mmol/L citrate buffer (pH 6.0), we incubated them for one hour with primary antibody, and the antibodies were localized by subsequent

incubation of a biotinylated secondary antibody and by Histostain-SP kit (Zymed Laboratories). The color in all immunostaining procedures was cultivated by diaminobenzidine. Sections were lightly stained with Harris' hematoxylin and mounted with Eukitt (Kindler, Freiburg, Germany). Non-neoplastic kidney, breast, skin, and liver samples served as positive and negative controls. Negative control staining was executed by substituting non-immune rabbit or mouse serum and phosphate-buffered saline for the primary antibodies.

We compared the immunostaining intensity of the tumor was compared to the normal glandular structures and scored them as follows: 0 = no immunostaining, 1 = less immunostaining intensity, 2 = same immunostaining intensity, 3 = more immunostaining intensity. Membrane-bound positivity was considered significant. The extent of immunostaining was assessed as follows: 1 = < 25%, 2 = 25-50%, 3 = 50-75%, 4 = > 75%. For easy quantitative analysis, we used the following descriptions: 1 = low, 2 and 3 = medium, 4 = high immunostaining. Two independent pathologists (L.E.B. Rosa and I.O. Bello) assessed and scored the sections, and discrepant cases were reanalyzed for consensus.

4.1.3 Study III

The aim of the study was to analyze the incidence, management, and outcome of pediatric patients with SGC.

The medical records of patients 19 years of age or younger and diagnosed with SGC between 1992 and 2011 were retrieved from the Departments of Otorhinolaryngology - Head and Neck Surgery and Pathology at the five University Hospitals in Finland. Ten patients with adequate available data were identified, and retrospective information included patient demographics, clinical and histological characteristics, and treatment and survival factors. Assessment of treatment and outcome was based on surgical, pathological, and radiological reports. The WHO classification and AFIP recommendations served in diagnostics and tumor grading. One boy had an MEC tumor originating from the ceruminous glands of the ear canal. Because of the histopathological similarity of the tumor and itsmanifestation in the periparotid location, we included him in the study.

The series comprised six girls and four boys with a median age of 14 years (range, 9-19 years) at diagnosis. The length of follow up was 7 months to 14 years.

4.1.4 Study IV

The aim of the study was to identify potential familial pedigrees of SGC and to recognize possible hereditary SGC cases in Finland with a population-based study in the Hospital District of Helsinki and Uusimaa.

The retrieval of the pathological files from the Department of Pathology, Haartman Institute, Helsinki University Central Hospital yielded 437 patients diagnosed with SGC between 1974 and 2009. Due to the long time retrieval period, the fact that SGC patients are older, and that these tumors carry a relatively poor OS, many were deceased at the time of the study. Consequently, we could reach only 161 patients from this cohort. We sent a detailed questionnaire to these patients about their physical health, relatives with malignancies, number of siblings, maternal and paternal aunts and uncles, and birthplaces of their grandparents. The questionnaire was sent in a prepaid, preaddressed envelope, and twice if necessary. Patients were contacted by phone or email to obtain more information, as long as they provided their written consent in the questionnaire. The total number of respondents was 88 (55%).

We included parents, siblings, aunts, and uncles in the analysis of cancer history but excluded grandparents because their possible cancer history is poorly known or undocumented.

The median age for those patients who completed the questionnaire was 57 years for MEC patients (range, 27 to 85), 50 for ACC patients (range, 19 to 77), and 59 for AdCC patients (range, 26 to 81). For all other patients with similar histological diagnoses retrieved from the database, the median age was 66 years (range, 15 to 87), 62 (range, 11 to 84), and 55 (range, 27 to 88). The number of MEC, ACC, and AdCC patients in the study group was comparable to patients with similar histology in the total database patient population of 437.

4.2 Statistical methods

In Study I, we used the nonparametric Kaplan-Meier method to estimate OS and DFS as a function of follow-up time and compared them to results of the nonparametric log-rank x^2 test. We conducted the statistical analyses using the SPSS software application for Windows (version 12.0.1, Chicago, IL, USA).

In Study II, we used Kaplan-Meier survival analysis to evaluate the statistical association between the results of the immunohistochemical analysis and patient survival. We then verified the survival results with Cox proportional hazards univariate regression models by using the extents and intensities for claudins. We used logistic regression models to compare survival plots over staining categories for claudins and SPSS version 16.0 in the statistical analyses. For the statistical analysis, we combined IMG-MEC and HG-MEC to which we compared LG-MEC.

In Study IV, we used the Kruskal-Wallis one-way ANOVA test to test the association between different SGC histologies and other site-specific malignancies in index patients' relatives.

Statistical results were considered significant when $p < 0.05$.

4.3 Ethics

The Research Ethics Board of the Helsinki University Central Hospital approved all the studies in this thesis (Dnro 410/E9/05, Dnro 33/13/03/04/2009, Dnro 31/13/03/02/2010, Valvira Dnro 425/05.01.00.06/2009). In Study IV, respondents approved their participation in the study by completing a written consent form. All the other studies were retrospective with no need for informed consent.

5 RESULTS

5.1 Characteristics and outcome of MEC (Study I)

The study comprised 23 LG-MECs, 7 IMG-MECs, and 20 HG-MECs. Patient characteristics appear in Table 7. The majority (75%) of the patients had cN0, whereas half of the patients with HG-MEC had cN+. The T stage differed across grades: T3 or T4 tumors were identified in 13% of LG tumors, in 29% of IMG tumors, and in 60% of HG tumors. Treatment consisted of surgery in 94% of cases and postoperative RT in 46% of cases (Table 8). For patients with cN0, 56% (22/39) received no therapy for the neck. As many as 23% (3/13) of patients with cN+ underwent ND, 46% (6/13) underwent ND and RT and 8% (1/13) underwent only RT. Three (23%) patients received palliative therapy for the neck due to patient desire or to widely spread disease. At presentation, four men with HG-MEC had distant metastases, which occurred in the mediastinum, lungs, bones, liver, or skin.

Table 7. Patient characteristics (modified from Study I).

Patient characteristics	LG, n = 23	IMG, n = 7	HG, n = 20	X, n = 2	Total, n = 52
Men	6	2	13	0	21
Women	17	5	7	2	31
Median age (years)	50	44	61	80	54
Range (years)	27-79	25-73	36-84	77-82	25-84
Tumor site					
Parotid gland	21	6	18	2	47
Submandibular gland	2	1	2	0	5
T-status					
T1	13	3	2	0	18
T2	7	2	6	1	16
T3	1	1	7	0	9
T4	2	1	5	1	9
N-status (%)					
N0	100	57	50	100	75
N+	0	43	50	0	25
Tumor stage					
I	13	3	2	0	18
II	7	0	2	1	10
III	4	3	1	0	8
IV	2	1	12	1	16
Patients with postoperative radiation (%)	9	86	80	0	46

LG = low grade; IMG = intermediate grade; HG = high grade; X = grade not determined; n = number

Table 8. Treatment modalities according to tumor grade (modified from Study I).

Grade	Sx, (%)	Sx + RT, (%)	No treatment, (%)
Low grade	91	9	0
Intermediate grade	14	86	0
High grade	15	80	5
Grade not determined	0	0	100
Total; n	25	24	3

Sx = surgery; RT = radiotherapy; n = number

The three-year OS was 72% for the whole patient series, 95% for LG tumors, 67% for IMG tumors, and 55% for HG tumors. In the minimum follow-up period of three years, patients with HG-MEC developed locoregional failures or distant metastases in 45% of cases, whereas patients with IMG-MEC developed locoregional failures in 33%, and distant metastases in 50% of cases. Patients with LG-MEC experienced no recurrences during follow up. Deaths from MEC occurred in 45% of the HG group, and in 33% of the IMG group. The three-year DFS was 71% for the whole patient series, and 100% for LG-MEC, 33% for IMG-MEC, and 55% for HG-MEC. We observed a statistically significant difference between LG-MEC and IMG-MEC or HG-MEC, but not between IMG-MEC and HG-MEC. Figures 4 and 5 illustrate the three-year DFS by grade and stage.

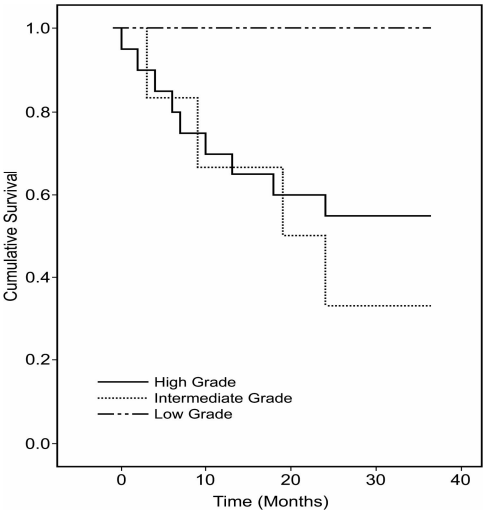


Figure 4. Three-year DFS by grade (modified from Study I).

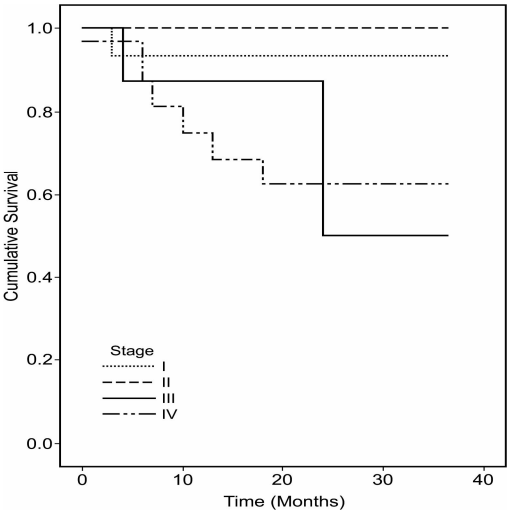


Figure 5. Three-year DFS by stage (modified from Study I).

5.1.1 Treatment and outcome of IMG-MEC (Study I)

We had seven patients with IMG-MEC. Table 9 shows the characteristics of this patient group. Six of these patients underwent FNAB. The sensitivity of FNAC in IMG-MEC was 50%. Patients with IMG-MEC in our patient series underwent surgery and postoperative RT in 86% of cases. The T stage in this group was T3 or T4 in 29% of cases. For comparison, LG tumors were T3 and T4 in 13% of cases. Cervical neck node metastases (cN+) occurred on first presentation in 43% of IMG-MEC patients who underwent ND. Local, locoregional, or distant metastases occurred in 71% of patients. Two patients died of disease during follow up.

Table 9. Characteristics of patients with IMG-MEC (Study I).

Gender	FNAC	TNM	Stage	Size of the tumor (mm)	Treatment	Site and time to recurrence (months)	Status at last follow up
Female	Benign neoplasia/ atypia	T1N0M0	I	7	Superficial parotidectomy	Scar; 6	AWD
Male	MEC?	T2N1M0	III	35	Total parotidectomy, MRND, RT	Ipsilateral neck; 9	NED
Female	Normal cytology	T3N0M0	III	15	Superficial parotidectomy, RT	-	NED
Female	Normal cytology	T1N0M0	I	8	Radical parotidectomy, RT	-	NED
Female	Not analyzed	T4aN1M0	IVA	4	Radical parotidectomy, RT	Lung; 24	DOD
Female	Atypia suspicious for malignancy	T1N0M0	I	12	Radical parotidectomy, RND, RT	Scar; 3 Skull base; 24	DOD
Male	Oncocytoma?	T2N1M0	III	21	Resection of submandibular gland, MRND, RT	Ipsilateral neck, axillary nodes; 31	AWD

FNAC = fine needle aspiration cytology; MRND = modified radical neck dissection; RND = radical neck dissection; RT = radiotherapy; AWD = alive with disease; NED = no evidence of disease; DOD = died of disease

5.2 Predictive value of immunohistochemical studies (Study II)

We analysed the expression patterns of claudins 1, 3, 4, and 7 in 39 patients with MEC of salivary gland origin. In IMG- and HG-MEC, staining characteristics for claudin-1 were only weak. In contrast, in LG-MEC, mostly membranous, but strong staining in cells of intermediate morphology was apparent. Adjacent normal salivary gland tissue served as a control for staining characteristics. Almost no staining for claudin-3 occurred in LG tumors, although it was strong and membranous in IMG and HG tumors. No clear benefit for differentiating LG and HG tumors resulted from the staining reactions for claudins 4 and 7.

The intensity for claudin-1 served to correctly categorize tumors into grades LG versus IMG and HG in 90% of cases. The importance of the extent of staining for claudin-1 was less important in grading. The higher the staining intensity for claudin-1, the higher the likelihood of an LG tumor. The intensity of claudin-3 staining correlated with histopathological grade in 72% of cases. High values portended an HG tumor with worse prognosis. The statistical differences for staining intensity and the extent for claudins 4 and 7 were less or not significant. The three-year DFS by immunostaining intensities for claudins 1 and 3 showed statistical significance (see Figures 6 and 7). The three-year OS was 74% and DFS 74% for the whole

patient series. The three-year DFS according to grade was 100% for LG-MEC, 50% for IMG-MEC, and 53% for HG-MEC.

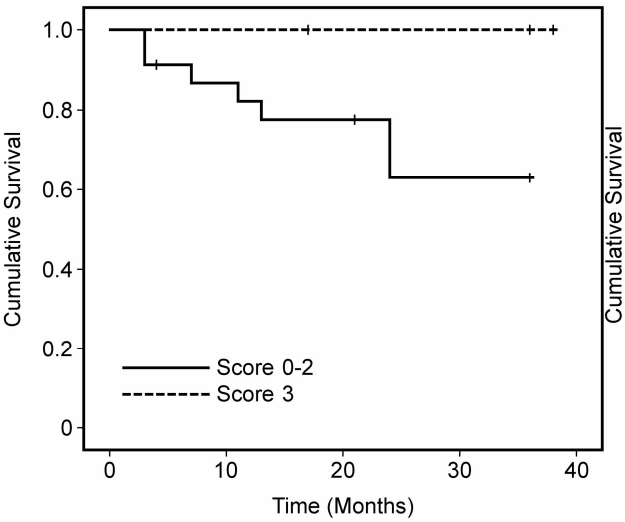


Figure 6. Three-year DFS by claudin-1 immunostaining intensity.
Score 0-2 = no immunostaining, less or same immunostaining intensity;
Score 3 = more immunostaining intensity.

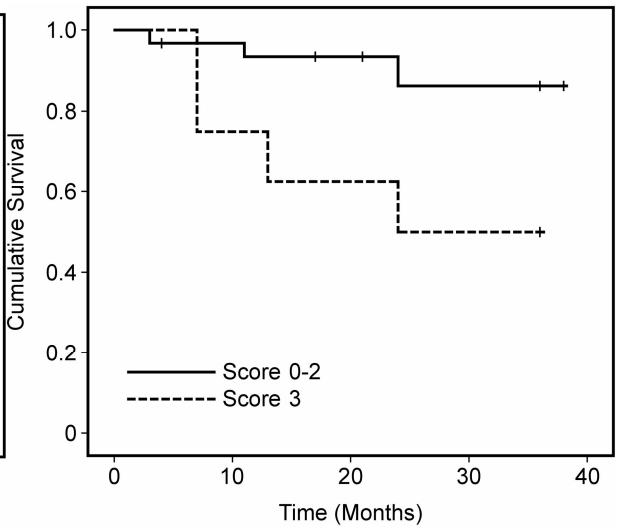


Figure 7. Three-year DFS by claudin-3 immunostaining intensity. Definition of scores appear as in Figure 5
(Figures 6 and 7 modified from Study II).

5.3 Pediatric salivary gland cancer in Finland (Study III)

Five pediatric patients with LG-MEC, four with ACC, and one with cystadenocarcinoma of salivary gland origin comprised the study group. Two patients had a history of malignancy prior to SGC in childhood. A 13-year-old boy had acute lymphoid leukemia four years prior to the MEC diagnosis. A 14-year-old girl had bilateral Wilm's tumor 13 years prior to the MEC diagnosis. Most of the tumors originated from the parotid gland (7/10), and two from the minor salivary glands of the lips. Additionally, one boy had an LG-MEC originating from ceruminal glands of the skin of the ear canal extending to a main tumor mass in a periparotid location. Surgery with curative intent was the primary treatment modality for all. Superficial parotidectomy was the operating procedure in most of the parotid gland tumors (5/7), and total parotidectomy in two cases (one Stage I ACC of the parotid gland, one Stage I MEC of the parotid gland). Tumors of the lips were resected. One Stage I ACC patient underwent elective ND because the initial surgery had inadequate surgical margins. One patient received postoperative RT because the tumor was originally classified as myoepithelial carcinoma but was eventually LG-MEC. The follow up ranged from 7 months to 14 years. No recurrences, metastases, or deaths occurred during this period, and all patients were disease free at the end of the follow up.

5.4 Heredity of salivary gland cancer in Finland (Study IV)

The study comprised 88 patients, of whom 66 had parotid gland carcinoma, 14 had submandibular gland carcinoma, 6 had minor salivary gland carcinoma, and 2 had carcinoma of an unspecified origin. The most common histologies were ACC (25%), MEC (24%), and AdCC

(20%). Patients reported an equal number of female and male relatives, and no gender difference existed in the relatives' cancer histories.

Of the MEC patients' relatives, 14% reported a history of cancer, compared to 5% for ACC, and 6% for AdCC. The relatives of MEC patients had more liver and prostate carcinomas than did the relatives of ACC and AdCC patients. In contrast, breast cancer was more common among AdCC and ACC patients' relatives than among MEC patients' relatives. These results did not reach statistical significance, however. Only one patient with parotid gland ACC reported a maternal aunt with SGC (Figure 8).

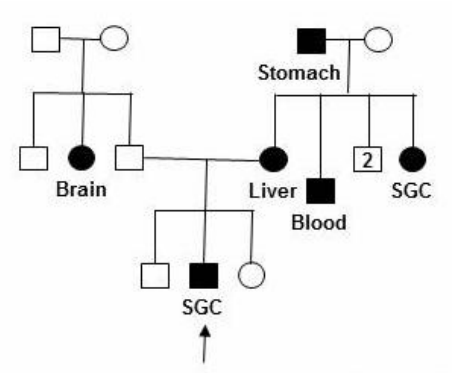


Figure 8. Pedigree of an SGC patient with a relative with SGC (modified from Study IV).

6 DISCUSSION

The expansive biological differences, rarity, and occurrence of SGC in all age groups make it a challenge to diagnose and treat. The outcome of MEC patients differs markedly according to the grade. LG-MEC tumor patients respond favorably to single modality treatment, which is often restricted to surgery. HG-MEC tumors show highly aggressive biological behavior with a tendency to recur, metastasize, and cause death. It is therefore of the utmost importance to diagnose MEC and to discover additional tools to grade MEC cases accurately (I). Treatment recommendations for and long-term outcomes of IMG-MEC remain controversial (I). Immunostaining intensities for claudins 1 and 3 help in distinguishing LG-MEC from IMG and HG (II). The claudins did not, however, reach significance over tumor grade in predicting DFS. MEC is also the most common subtype in pediatric SGC patients, resulting in favorable outcomes without recurrences, metastases, or deaths during follow up (III). The possibility of hereditary forms of cancer is important. One SGC patient reported a maternal aunt with SGC, but no cases occurred among first-degree relatives (IV).

6.1 Study limitations

Because SGC is rare and tumors show considerable histopathological diversity, research on this topic is difficult. Moreover, in Studies I, II, and III, patients were few. These cases, however, were representative of the incidence and prevalence of SGC, since the treatment of head and neck malignancies in Finland is centralized in the five University Hospitals. All the patient information was collected from the files of these tertiary referral centers. Study I had only five submandibular gland MECs and seven IMG-MECs. This small cohort size may have impacted the results and conclusions of the study. Further, the patient population covered a long time period, and for some (8/60 in Study I) patients, no follow-up data could be obtained. This limited the average follow up to only three years for the DFS analysis in Studies I and II. In Study II, 39 of 60 patients had sufficient tumor material and clinical data.

According to the Finnish Cancer Registry, 15 pediatric SGC patients were diagnosed in Finland from 1990 to 2009. In Study III, sufficient data were unavailable from 5 of 15 cases. Furthermore, in that study, the time period differed and excluded the years 1990 and 1991. We chose the time period from 1992 to 2011 because the diagnostic designations of MEC and ACC were first introduced to the WHO classification as true malignancies in 1991. Consequently, the series comprised only 10 cases. The proportion of malignancy in all pediatric salivary gland tumors in Finland remains unknown, since only malignant cases were included in the study. Nevertheless, no previous reports exist on pediatric SGC in Finland, so our cases are representative of the national status of these diseases.

In Study IV, the clinical data were based on hospital records, but information on the relatives affected was provided mainly by the index patient with SGC. We carried out the study using a questionnaire. Since patients diagnosed with SGC after 1974 were included in the retrieval, many were already deceased at the time of the analysis. However, the distribution of the most common SGC histologies was similar among the participants and non-participants. Also, the age at diagnosis was similar among both the participants and non-participants. Of the 161 SGC patients identified who were alive and who could be contacted, 88 (55%) returned a completed questionnaire. Only after they provided their written consent was contact by phone or email possible. Grandparents were excluded from the analysis of cancer history due to the fact that their exact medical history could not be well remembered and/or documented. SGC patients' children were excluded from the analysis because they had not reached the age when general cancer risk in the population is higher. None of the SGC patients in the present study reported having children with SGC.

6.2 General characteristics of MEC patients (Study I)

Some studies have reported a female preponderance in certain MEC series (Eveson 2011, Thompson 2005), and Study I showed a similar tendency. In addition, all patients with distant metastasis at presentation were men, and still others have found differences between sexes in DFS as well (McHugh et al. 2012). The reason for this difference is likely multidimensional. Non-tumor-related factors most likely include age, differences in lifestyle, and comorbidities. Almost all tumors were located in the parotid gland (90%) (I), which is also a common finding in other studies on MEC (Goode et al. 1998, McHugh et al. 2012).

6.3 Tumor grade influences prognosis of MEC (Study I)

In 35% of cases, tumors were T3 or T4, and most (67%) were HG. Tumors over 4 cm in size are at 20% risk for occult metastases (Armstrong et al. 1992), and tumor grade also influences this risk. HG-MEC is at 57% risk for occult metastases, IMG-MEC 17%, and LG-MEC 11% (Regis De Brito Santos et al. 2001). Nance and colleagues (Nance et al. 2008) reported cN+ disease in 32% of their cases (this occurred in 25% of our cases), but none in LG-MEC. Four (8%) of our patients exhibited distant metastases at presentation, and metastases occurred during follow up in 40% of HG-MEC, and 29% of IMG-MEC cases. McHugh and colleagues (McHugh et al. 2012) had 5 (4%) patients with distant metastasis at initial presentation, and 13 (11%) developed distant metastasis during follow up.

RT was administered for HG tumors in 80%, IMG tumors in 86%, and LG tumors in 9% of cases. McHugh and colleagues (McHugh et al. 2012) reported that in their series patients underwent RT more often for IMG-MEC tumors than for LG-MEC. Clearly, the former presented more often with positive surgical margins, vascular or perineural infiltration, or widely spread disease. These HG features resulted in more aggressive treatment, portending a higher survival rate that showed no difference from that of LG-MEC patients.

An evaluation of the treatment modalities and patient outcomes for major salivary gland MEC revealed notably poor outcomes for IMG-MEC patients. The unpredictable biological behavior of IMG-MEC is evident in our patient population (I). The recurrence rate was high among these patients. Two patients with IMG-MEC developed distant metastases during follow-up, and patients with IMG-MEC had only a three-year DFS of 33%. In a localized T1 parotid gland IMG-MEC, superficial parotidectomy proved inadequate and resulted in tumor recurrence. In a similar IMG-MEC case, a patient was cured with radical parotidectomy combined with adjuvant RT. On the other hand, even the radical type of surgery in Stage I parotid gland IMG-MEC could not avoid distant metastasis and death from disease. The biological behaviour of these tumors resembled HG-MEC more than LG-MEC.

We observed a statistically significant difference in DFS by grade between LG-MEC and IMG- or HG-MEC. Patients with LG-MEC experienced no recurrences, metastases, or deaths from disease during follow up, but a dramatic decrease in survival was evident in IMG- and HG-MEC. DFS in these two latter groups showed no statistically significant difference. In contrast with these results, others combine IMG- and LG-MEC in the same category because they show similar survival rates (McHugh et al. 2012, Nance et al. 2008). Nance and colleagues (Nance et al. 2008) reported locoregional recurrences in 30% of HG-MEC, 23% of IMG-MEC, and none of LG-MEC patients. In their series, death from disease occurred in 52% of HG-MEC, but no IMG-MEC patients died from their cancer.

The primary treatment of submandibular gland tumors should be aggressive. Goode and colleagues (Goode et al. 1998) studied 234 SGCs of major salivary gland origin to evaluate

their own grading criteria in this series. Patients with submandibular gland MEC died from disease in 13% of cases (4/31), and 75% (3/4) of these tumors were histologically LG. In contrast, 62% (13/21) of deaths due to parotid gland MEC were among patients with an HG tumor. They conclude that the biological course of submandibular gland tumors is difficult to predict on the basis of tumor grade. In their opinion, patients with submandibular gland MEC should be treated aggressively regardless of grade and closely monitored after treatment. We had only five submandibular gland MECs, and their outcomes showed no difference from those of parotid gland tumors with similar histologies.

6.4 Claudins in MEC (Study II)

Grading MEC is difficult, and clear auxiliary immunohistochemical staining parameters are lacking. Consequently, we explored the role of claudins in grading MEC (II). No previous studies concerning claudins in SGC exist. In salivary gland MEC, the staining characteristics for claudins 1, 3, 4, and 7 varied according to grade. Claudins 1 and 3 showed statistical significance for grading MEC. Thus, the immunohistochemistry for claudins 1 and 3 can serve as a possible auxiliary tool for grading MEC. Of all the claudins we studied, intensity for claudin-1 appeared to be the best predictive parameter for MEC. In comparison with the AFIP grading system in routine histopathology, the intensity for claudin-1 defined the tumor grade correctly in 90%. The tumor was almost always LG when claudin-1 intensity was highest, and these patients experienced no recurrences. IMG- and HG-MEC evidently show similar staining characteristics for claudin-1. Patients with IMG- and HG-MEC experience recurrences, metastases, and deaths from disease. IMG- and HG-MEC showed higher immunostaining intensity for claudin-3, corresponding to worse prognosis. Compared to histopathological grades, the intensity for claudin-3 defined tumor grade correctly in 72% of cases. Of all the claudins we studied, we found no statistically significant differences between IMG- and HG-MEC in immunostaining for claudins.

The claudins studied did not achieve significance over tumor grade; they therefore influence survival indirectly through the tumor grading. The immunostaining characteristics for claudins 1 and 3 help in distinguishing LG from IMG or HG, and the distinction between these two extremes yields clear differences in outcome. Dos Reis and colleagues (Dos Reis et al. 2008) showed that claudin-1 overexpression associates with increased invasiveness and poorer survival in oral SCC, although others did not support this finding (Bello et al. 2008). Tongue SCC strongly expresses claudins 1 and 7, the latter of which showed significance for survival (Bello et al. 2008). A previous study reported that claudin-7 is downregulated in head and neck SCC (Al Moustafa et al. 2002). Claudins are tumor-type specific, and the same claudin shows various expressions in different malignancies. The level of immunostaining for claudins also influences survival (Bello et al. 2008). Future studies are needed to identify how external signals can modulate claudins and tight junctions. The search for specific markers will help to identify targets for SGC therapy as well as functional aspects in benign conditions with hyposalivation (Baker 2010).

The three-year DFS of LG and IMG or HG differed markedly. Because our study shows the biological behavior and immunostaining characteristics for claudins in IMG- and HG-MEC to be similar, IMG-MEC in our patient cohort appears to differ from LG-MEC. Treatment for IMG-MEC should be aggressive and comparable to HG-MEC.

6.5 Management and outcome of pediatric salivary gland cancer in Finland (Study III)

Pediatric SGC is a rare group of malignancies. Because the incidence of SGC increases with age the mean age of pediatric SGC patients is 14 (Kupferman et al. 2010, Ryan et al. 2011) (and also Study III). All pediatric SGCs in our cohort had LG tumors, and all had favorable outcomes without recurrences or metastases. This is in accordance with Clode and colleagues (Clode et al. 1991), who reported that six LG-MECs remained disease-free after a five-year follow up. In their series, two primarily extraglandular recurrent cases experienced no complications after a second surgery. This emphasizes the fact that recurrences can also be managed surgically without worsening survival in this patient population.

In the pediatric patient group, parotid gland tumors prove malignant in 50% of cases (Guzzo et al. 2006). Preoperative treatment decisions concerning the surgical approach are difficult because FNAC is inaccurate in diagnosing salivary gland malignancy (Eveson 2011, Atula et al. 1996). In our pediatric SGC patient series, none of the cytological specimens examined (7/10) proved suspicious or confirmed malignancy. All these tumors were LG, which diminishes the sensitivity of FNAC. The sensitivity of FNAC to malignancies improves with grade. Superficial parotidectomy should be the standard operative procedure in all pediatric parotid gland lesions localized to the superficial lobe of the gland to evaluate intraparotid nodal status and to stage tumors accurately. According to this management algorithm, reoperations at elevated risk for dysfunction of the facial nerve may be avoided. If a malignancy is evident, total parotidectomy is recommended. Surgery is usually an adequate treatment modality in pediatric SGC, provided that it is sufficiently aggressive to gain negative surgical margins.

Elective ND is most often unnecessary for pediatric SGC, as most tumors are LG and localized to the primary site, and occult metastases are rare in LG lesions (Ribeiro Kde et al. 2002, Sultan et al. 2011). One of our patients underwent ND due to positive surgical margins, but RT was avoided because the tumor was of LG malignancy (III). Although the treatment modalities in our study varied, RT was seldom administered, as adjuvant RT for pediatric SGC patients is recommended in invasive disease, in tumors with HG features, and in cases of positive surgical margins (Orvidas et al. 2000). Also, the higher risk for a second malignancy in the irradiated field is worth considering (Kupferman et al. 2010). Another patient received RT because the tumor was first diagnosed as myoepithelial carcinoma (but turned out Stage I MEC), which has a higher potential to recur locally (III).

HG tumors are infrequent in the pediatric population. Védrine and colleagues (Védrine et al. 2006) had two (2/16; 13%) pediatric HG-MECs, as did Hicks and Flaitz (Hicks et al. 2000) (2/26; 8%). Three of these patients died from disease within four years of treatment. Some of these HG-MECs may represent other HG malignancies. This tends to confuse the survival analysis of MEC and comparison between studies. Similarly to adults, pediatric HG tumors tend to have locoregional metastasis at presentation and to recur during follow up (Ryan et al. 2011). No HG tumors were present in our cohort.

The length of follow-up time varied from 7 months to 14 years. No recurrences occurred. Two patients had a previous malignancy 4 and 13 years before their diagnoses of salivary gland MEC. One patient had acute lymphoid leukemia, and the other, Wilm's tumor; both underwent chemotherapy. The prior cancer treatment did not worsen their survival after SGC, and no evidence of either cancer was observed in these two patients after their treatment for SGC. Compared to patients with MEC as a primary, MEC, as a second primary has no influence on survival (Védrine et al. 2006). Our results concur with those in the literature: the five-year OS of pediatric SGC patients is > 90% (Sultan et al. 2011, Guzzo et al. 2006, Kupferman et al. 2010).

According to the literature, MEC is the most common SGC subtype also in pediatric patients (Ribeiro Kde et al. 2002, Sultan et al. 2011, Kupferman et al. 2010, Ryan et al. 2011); our Finnish patient cohort (III) also supports this finding. No previous studies have evaluated the incidence, management, and outcome of pediatric SGC in Finland. Our patient cohort must be followed up for many years to obtain information on the long-term outcome of pediatric SGC patients. Because SGC a rare group of diseases, experience in its treatment should originate primarily from treatment of adults with similar diagnostic tumor entities. Our study did not show the proportion of malignancies in pediatric salivary gland tumors. According to the literature, pediatric salivary gland tumors are malignant in 35 to 50% of cases, and half of parotid gland tumors are malignant (Ribeiro Kde et al. 2002). Most pediatric SGCs occur in the parotid gland (87%); occurrences in other sites are few (Sultan et al. 2011). Unequivocal treatment recommendations are therefore difficult to establish. Nevertheless, all pediatric salivary gland tumors should be considered as malignancies until proven otherwise.

Patients in Study III had no first-degree relatives with SGC, an observation that will also enjoy confirmation after a longer follow up.

6.6 Familial predisposition for salivary gland cancer in Finland (Study IV)

The possible inheritance of cancer is an important issue for all cancer patients and their relatives. Because SGC affects patients in all age groups, and even pediatric cases exist, speculation exists about a possible genetic background for SGC. Screening the family history of SGC patients thus seemed warranted, so we performed screenings in Study IV. More importantly, we studied familial predisposition for SGC in Finland with a population-based approach in the Hospital District of Helsinki and Uusimaa because of the genetic uniqueness of the Finnish population. The small number of original founders followed by the population's geographical and cultural isolation restricted the Finnish gene pool (Peltonen et al. 1999). Thus, any conclusions about familial predisposition for SGC in the Finnish population can be based only on studying Finnish SGC patients and their relatives. Also, no previous studies have explored the heredity of SGC in Finland. In our study of 88 respondents, 1 male patient with parotid gland ACC reported a maternal aunt with SGC, but no affected individuals had first-degree relatives with SGC. Other studies concur with our findings on of which reported the proportion of familial SGC in Sweden as 0.15% (Hemminki et al. 2008). For comparison, familial breast cancer in that same study constituted 14%. Although an association between SGC and sequential breast cancer may exist, studies have found no links to breast cancer (In der Maur et al. 2005). In our patient cohort, three (5%) patients had sequential breast cancer, the most common malignancy among women in Finland with an age-standardized incidence rate of approximately 95 per 100 000 person-years (Finnish Cancer Registry 2012).

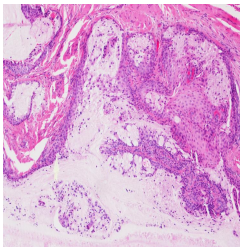
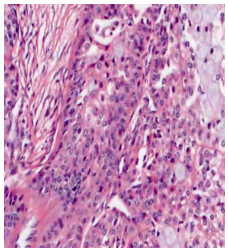
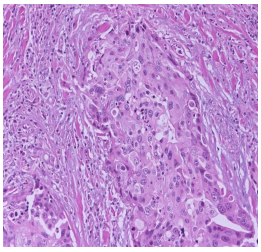
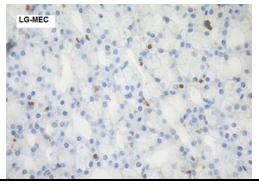
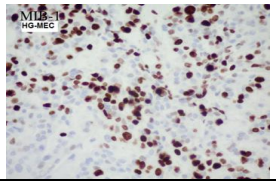
Undifferentiated SGC is associated with EBV infection and shows similar histopathology with NPC. A genetic and environmental element explains why Inuits in Greenland experience up to 9-fold higher incidence of lymphoepithelial-like SGC (Friborg et al. 2008, Albeck et al. 1993). Their risk for SGC remains unchanged after migration, which emphasizes the underlying genetic aspect of the disease (Friborg et al. 2008). Two studies report familial parotid gland ACC. One case occurred in a family in which the father and daughter had ACC, and the other case occurred in a family in which the brother and sister had ACC (Delides et al. 2005, Depowski et al. 1999). In our limited patient population, no clear hereditary background for SGC and no site-specific association between SGC and other malignancies existed. We were unable to identify any first-degree relatives with SGC. The Finnish Center of Excellence in Cancer Genetics Research (http://www.helsinki.fi/coe/cancer-genetics/index.html) has

screened approximately one million cancer cases in Finland for familial predisposition of SGC among various malignancies. In accordance with the present results, they found no familial clustering of SGC in the Finnish population.

6.7 Perspectives of MEC

LG-MECs are almost always easy to recognize with conventional histopathology. Brandwein and colleagues (Brandwein et al. 2001) concluded that the AFIP grading scheme tended to downgrade MEC. The point-based grading of MEC according to AFIP does not include invasion, and a difference of one single point separates IMG from HG (Auclair et al. 1992, Goode et al. 1998). At a speculative glance, some tumors classified as IMG-MEC according to the AFIP grading scheme should therefore be classified as HG-MEC. Downgrading the histological diagnosis could theoretically be considered a possible causative factor of the notably poor outcomes among the IMG-MEC patients in our study (I). Also, the differential diagnosis of HG-MEC from other HG malignancies seems to be of the utmost importance. The immunostaining intensity for claudin-3, MUC5AC staining characteristics, and MECT1-MAML2 translocation status could prove useful in the differential diagnosis of HG-MEC. Further, knowledge of the genetic and molecular alterations in SGC deepens our understanding of the biology of SGC. Thus, improving both the classification system and the available treatment modalities should be the main goals in this field. Table 10 summarizes the characteristics of salivary gland MEC based on the present results and the existing literature on adult cases.

Table 10. Characteristics of salivary gland MEC.

	LG-MEC	**IMG-MEC**	**HG-MEC**	Reference
Histology				Courtesy of Professor Ilmo Leivo
Incidence	16-32% 44% (Study I)	44-51% 14% (Study I)	23-35% 39% (Study I)	Luna 2006, Study I
Proliferation markers	MIB-1 index < 10% 		MIB-1 index > 10% 	Skalova et al. 1994, illustrations courtesy of Professor Ilmo Leivo
Biological markers	MECT1-MAML2 translocation present in up to 70%		MECT1-MAML2 translocation present in up to 50%; EWSR1-POU5F1 translocation association; MECT1-MAML2 negativity + HMGA2 expression ↑	Martins et al. 2004, Okabe et al. 2006, Moller et al. 2008, Fehr et al. 2009

Claudins ↔ = no difference in expression; ↑ = elevated expression	Claudin-1 ↑ Claudin-3 ↔	Claudin-1 ↔ Claudin-3 ↑	Claudin-1 ↔ Claudin-3 ↑	Study II
Mucins, percent (%) of tumors with staining for: - MUC1 - MUC2 - MUC4 - MUC5AC	 71 28 91 78	 60 30 80 60	 76 0 50 53	Handra-Luca et al. 2005
Preoperative examinations	US + FNAB + MRI or CT	US + FNAB + MRI or CT - thorax CT if cN+, or suspicion of M1	US + FNAB + MRI or CT - thorax CT if cN+, or suspicion of M1	
Initial treatment recommendations; see Table 5 - FSs in all cases (not accurate) - ND in selected cases	Sx (total parotidectomy, total resection of the gland)	Sx + RT in selected cases	Sx + RT Chemotherapy in selected cases	Seethala 2011, Klussmann et al. 2008, Nance et al. 2008
Survival (%) - 5-year DFS* - 3-year DFS (Study I)	 90-100 100	 90 33	 30-40 55	*McHugh et al. 2012, Healey et al. 1970, Study I

US = ultrasonography; FNAB = fine needle aspiration biopsy; FS = frozen section; ND = neck dissection; Sx = surgery; RT = radiotherapy; DFS = disease free survival

7 CONCLUSIONS

1. Patients in the present series with LG-MEC tumors always had cN0 and most (91%) of them were treated primarily with surgery. These patients experienced no recurrences, metastases, or cancer-related deaths during follow up. IMG-MEC exhibits similar biological behavior to that of HG-MEC. IMG-MEC patients presented with cN+ in 43% of cases and also experienced distant metastases in 50% of cases during follow up. HG-MEC patients presented with cN+ in 50% of cases.

2. The immunostaining intensity for claudin-1 separated LG-MEC from IMG- and HG-MEC. IMG- and HG-MEC tumors were more likely to express higher staining for claudin-3 than were LG-MEC tumors. IMG-MEC tumor characteristics resembled those of HG-MEC in both immunohistochemical claudin analysis and survival analysis.

3. Pediatric SGC is rare and tumors usually present with indolent LG histology and are localized to the primary site. Long-term survival data are lacking, however, so to improve it, these patients should be followed up several years after treatment. Two children (20%) had a prior malignancy before MEC diagnosis which was treated with chemotherapy. The elevated risk for SGC as a second malignancy in the pediatric patient population, based on the present study and existing literature, is noteworthy.

4. Based on a limited questionnaire survey, familial predisposition for SGC in the Finnish population appears to be insignificant. Other surveys that have screened for possible hereditary SGC cases without positive results also support this finding.

5. Surgery seems to be an adequate treatment modality for LG-MEC. For IMG-MEC, according to our study showing a poor outcome for these patients, an aggressive treatment approach with a combined modality seems appropriate. An aggressive combined modality treatment is mandatory for all HG tumors. Because of the indolent nature of SGC among pediatric patients, these tumors can be managed primarily with radical surgery.

ACKNOWLEDGEMENTS

This study was conducted at the Department of Otorhinolaryngology – Head and Neck Surgery and the Department of Pathology, HUCH, between 2006 and 2012. The Cancer Society of Finland, the Finnish Society of Otorhinolaryngology, the Helsinki Central Hospital Funds (EVO), and the Finnish Medical Society Duodecim provided financial support.

I express my gratitude to the chief of the clinic group, Docent Hans Ramsay, and the head of the clinic, Docent Heikki Rihkanen, for providing an encouraging and friendly atmosphere to work in, as well as to Professor Anne Pitkäranta for serving as a role model for all academic achievements.

I am privileged to have had Professors Antti Mäkitie and Ilmo Leivo as my supervisors. Their enthusiasm for science is captivating, and their encouraging approach to this scientific project was invaluable.

I thank the official reviewers, Professor Heikki Löppönen and Docent Pasi Hirvikoski, for their valuable feedback and comments. I also thank my collaborators Tuula Salo, Ylermi Soini, Luiz Eduardo Blumer Rosa, Ibrahim Bello, and Reidar Grénman, as well as Kauko Saarilahti for his wise and practical advice. Especially warm appreciation goes to Tuomas Klockars, who turned out to be irreplaceable in this project. He always managed to make time for me, showing rational guidance and cheery encouragement.

I express my gratitude to all my friends and colleagues at our clinic and also at other departments. The excellent team spirit always helped through mentally and physically hard workdays - my warmest appreciation to Marjo Lindberg-Kaita! I look back on those numerous moments in all our units filled with laughter, jokes, and good company. I thank Mervi Kanerva and Riina Niemensivu with a smile, since you always made me smile when I needed encouragement, and Riitta Saarinen shared with me the struggles and glory of science. Leif Bäck, my idol. You have always believed in me and supported me in all new challenges (which you yourself often provided!). I hope never to let you down.

I cherish all my friends throughout life who have stayed with me all these years. Saila has always been like my third sister.

I thank my mother-in-law, Hillevi, and my grandparents-in-law, Raija and Pentti, for all their help in everyday life. And many warm thanks for those ready-cooked meals! My parents, Liisa and Heimo, and my sisters, Hannele and Kristiina, and her husband Joonas, have always been there for me and my family. I especially thank Aino and Elias for being the best cousins for our children and for keeping them company when I was preoccupied with this project.

I dedicate this work to my family. I thank my husband, Aapo, for his everlasting love and support. I adore our children, Matias and Aamos. Their lively, energetic, and constantly humoristic personalities guarantee joy in my life.

Helsinki, November 2012,
Katri Aro

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