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ORIGINAL ARTICLE

Differing Lymphatic Vessels Density in Salivary Adenoid Cystic Carcinoma and Pleomorphic Adenoma

Richard Salzman[1] · **I. Stárek**[1] · **L. Kučerová**[2] · **A. Skálová**[3]

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Abstract Benign and malignant tumours are known to express various factors inducing lymphangiogenesis. Despite their different biological behaviour, salivary pleomorphic adenomas (PA) and adenoid cystic carcinomas (SACC) show similar lymphatic network. Authors compare density of lymphatic network in these tumours. The retrospective study included 20 SACC and 20 PA from salivary tumours. Lymphatic vessel density (LVD) was identified using D2-40 antibody and counted. In SACC, intratumoral, respectively peritumoral, lymphatic vessels were identified in 100 %, respectively 93.8 %, of cases. The intratumoral and peritumoral LVD did not significantly differ from each other. However, they both were higher than normal parenchyma density. In PA, intratumoral LVD, with a single exception, revealed values of 0 and 1. The intratumoral was found to be lower than peritumoral density. The LVD in healthy gland, similar to peritumoral one, was significantly higher than intratumoral values. Direct comparison showed intratumoral and peritumoral LVD in PA to be lower than in SACC. This study comparing LVD in PA and SACC revealed higher values in SACC, outnumbering those in healthy salivary parenchyma and PA. It suggests the capability of this biologically aggressive neoplasm to induce lymphangiogenesis.

Keywords Adenoid cystic carcinoma · Lymphangiogenesis · Lymphatic vessel · Pleomorphic adenoma · Salivary gland

The first 2 authors contributed equally to the preparation of the manuscript.

✉ Richard Salzman
richard.salzman@fnol.cz

L. Kučerová
kucerlad@centrum.cz

A. Skálová
skalova@fnplzen.cz

[1] Department of Otorhinolaryngology, Faculty of Medicine and Dentistry, Palacky University Olomouc, University Hospital Olomouc, I. P. Pavlova 6, 775 20 Olomouc, Czech Republic

[2] Department of Clinical and Molecular Pathology, Faculty of Medicine and Dentistry, Palacky University Olomouc, University Hospital Olomouc, I. P. Pavlova 6, 775 20 Olomouc, Czech Republic

[3] Department of Pathology, Charles University in Prague, Alej svobody 80, 304 60 Pilsen, Czech Republic

Introduction

Lymphogenic metastasizing of malignant tumours is supposed to be due to the invasion of neoplastic cells in preexisting lymphatic network [1], present at the tumour periphery [2, 3]. In addition to this traditional concept, there is recently growing in both clinical and experimental evidence that many human carcinomas have capacity to express various lymphangiogenous factors thereby inducing formation of new lymphatic channels [4]). This process can be associated with lymph node metastasis, and consequently with poor prognosis [5].

Pleomorphic adenoma (PA) is the commonest benign salivary tumour [6], notorious for the propensity to recurrence. Since nodules of recurrent PA often invade soft tissues far from the primary tumour site, Stennert [7] suggests dissemination of its cells through lymphatic vessels. This raises the question whether recurrent PA might stimulate lymphangiogenesis. However, our previous study failed to demonstrate any significant difference in density of lymphatics in primary and recurrent PA [8]. Moreover, it revealed almost absent lymphatic vessels in the core (intratumoral lymphatic vessel density, iLVD), of all these tumours and

significantly higher density of lymphatics present at the tumour periphery (peritumoral lymphatic vessels density, pLVD), equalling to that in healthy salivary parenchyma. Our findings thus did not suggest the formation of lymphatic vessels in pleomorphic adenoma. Similar results and conclusions were reported by Fujita [9] in adenoid cystic carcinoma (SACC). This tumour differs from other high-grade salivary carcinomas in the low incidence of lymph node metastasis [10], reported not to exceed 10 % [11]. However, Woolger [12] noticed that the real incidence of lymph node involvement might be higher than previously thought. The reason for that could be minimal amount of desmoplastic stroma produced by this histopathologic entity. Therefore, the volume of affected nodes is kept at very low level, which makes them undetectable for a long time. The real capability of SACC to lymphatic spread is thus a matter of debate, deserving further research.

Similarity in the reported presence, distribution and density of lymphatic vessels in PA [8] and SACC [9] doesn't correspond to diverse biologic nature of these two entities. Therefore, in the presented study, the authors directly compare lymphatic network in PA and SACC, which share similar histogenesis, involving the participation of both ductal and myoepithelial neoplastic cells. Moreover, the results could be contributory for understanding of real potential of the latter tumour to lymphangiogenous spread.

Materials and Methods

Clinical Data

The retrospective study was performed on tissue samples from 20 patients (12 females and 8 males, age ranging from 24 to 84 years, mean 56.4+/−15.0 years) diagnosed with SACC, and other 20 tissue samples from 15 patients (13 females and 2 males, age ranging from 33 to 69 years, mean 53.5+/−12.4 years) diagnosed with parotid PA. The samples were retrieved from Tissue Archives of participating pathology departments.

In SACC, fourteen tumours originated from great (6 parotid, 6 submandibular, 2 sublingual) and 6 from small salivary glands (buccal mucosa and maxilla two each, hard palate and palatine tonsil one each). Seven, six, three and four were diagnosed at the clinical stage I, II, III and IV, respectively. Six patients presented with clinically positive neck nodes (cN+). Eleven and 9 tumours were histologically graded as G2 and G3, respectively, according to Szanto [13]. Sixteen out of 17 surgically treated patients received adjuvant radiotherapy alone or in combination with chemotherapy. The remaining patient was treated with surgery only. Radiation therapy and concurrent chemoradiotherapy were applied in one case each. One patient with locally very advanced tumour and distant metastasis refused further treatment after excision biopsy was performed. Three out of 6 N+ patients (one patient refused treatment and two received radiation therapy to N+ necks) received curative neck dissections. Three cN0 staged cases (with clinically suspected high stage parotid malignancies and pre- or perioperatively unknown diagnosis of SACC) underwent elective neck dissections. Complete remission (CR) was achieved in 18 out of 20 patients. The patients who didn't reach CR were excluded from survival analysis. The follow-up ranged between 5 and 286.1 months. Median survival was 46.7 months.

In PA, 10 primary and 10 recurrent tumours were analysed. All but two of 10 primary tumours originating from the superficial parotid lobe were removed by partial or superficial (4 cases each) parotidectomy. Two patients with deep lobe and parapharyngeal parotid tumour underwent total parotidectomy (with facial nerve preservation) and transoral exstirpation, respectively. Recurrent PA were removed by superficial (3 cases) or total conservative parotidectomy (7 cases).

Evaluation of Lymphatic Vascular Density

Sections of 4 μm thickness were cut from each archived paraffin embedded tissue sample throughout the whole specimen. Lymphatic microvessels in the tumour centre, on its periphery, and in healthy salivary parenchyma were identified through immunohistochemical staining using the D2-40 antibody (Fig. 1). For evaluation of both intra- and peritumoral LVD, the area of highest vascularization (hot spots) was identified on a 40× field, and the number of vessels per square millimetre was counted in a 200× field. The LVD was evaluated by two experienced pathologists (LK, AS). In discrepant findings, specimens were reviewed to achieve conformity. iLVD was assessed in all tissue samples. pLVD was evaluated only in 17 PA and 16 SACC with obvious tumour periphery (i.e. the capsule or margins of the tumour).

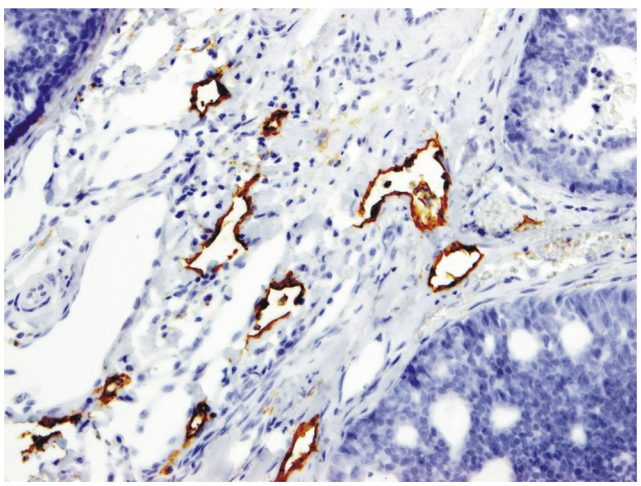

Fig. 1 Intratumoral lymphatic vessels in salivary adenoid cystic carcinoma. D2-40 positive staining

LVD was also quantified in normal parenchyma (nLVD) remote (≥1.5 cm) from the tumour in both tumour groups (15 tissue samples around PA and 12 around SACC).

Statistical Analysis

The Mann–Whitney *U*-test was used for comparison of the LVD between tumour groups. All analyses were performed with STATISTICA v. 10.0 (Statsoft Inc). A p value of <0.05 was considered statistically significant.

Results

Lymphatic Vessel Density in SACC

In SACC, intratumoral lymphatic vessels could be demonstrated in all tumours. Peritumoral lymphatics were seen in 15 out of 16 lesions (93.8 %).

The iLVD (min 1; max 15; mean 6.3 ± 3.8) and pLVD (min 0; max 15; mean 7.0 ± 4.0) values didn't significantly differ from each other (Mann–Whitney, $p > 0.05$, Fig. 2).

Peritumoral lymphatic vessels were mostly dilated (10 out of 15) in contrast to intratumoral vessels which were found to be mostly compressed (14 out of 20).

Lymphatic Vessel Density in PA

The iLVD, with a single exception, revealed values of 0 and 1 (min 0; max 2; mean 0.5 ± 0.6; median iLVD = 0). The pLVD values varied from 0 to 6 (mean 2.0 ± 1.9, Fig. 2). The iLVD was significantly lower than pLVD values (Mann–Whitney, $p = 0.01$).

Lymphatic Vessel Density in Normal Salivary Parenchyma

There was no difference between nLVD evaluated in SACC (range 0–8, mean 1.75 ± 2.56) and PA samples (range 0–5, mean 2.07 ± 1.16). In SACC, both iLVD and pLVD were significantly higher than nLVD ($p = 0.0009$ and $p = 0.0008$, respectively). In PA, iLVD and nLVD differed significantly ($p < 0.0001$). Our study showed no significant difference between pLVD and nLVD ($p > 0.05$, all Mann–Whitney, Fig. 3).

Comparison of LVD Between PA and SACC

The iLVD, respectively pLVD, was found to be significantly lower than in SACC (Mann–Whitney, $p < 0.01$ for both, Fig. 4).

Discussion

Lymphangiogenesis in salivary gland tumours has been paid minimal attention to so far. Our literature review revealed only three papers (including the one of ours) dealing with this issue in benign sialomas. In concert with our previous study [8], Soares [6] demonstrated minimal density of intratumoral and significantly higher density of peritumoral lymphatics in pleomorphic adenoma. Teymoortash [14] compared the density of lymphatic vessels in the normal parotid gland and intraparotid lymph nodes to that in pleomorphic adenoma and Warthin's tumour. The density of intratumoral lymphatic vessels in the former neoplasm was very low, as was that in the normal parotid and lymph nodes. However, all these values were significantly lower than the number of intratumoral lymphatic

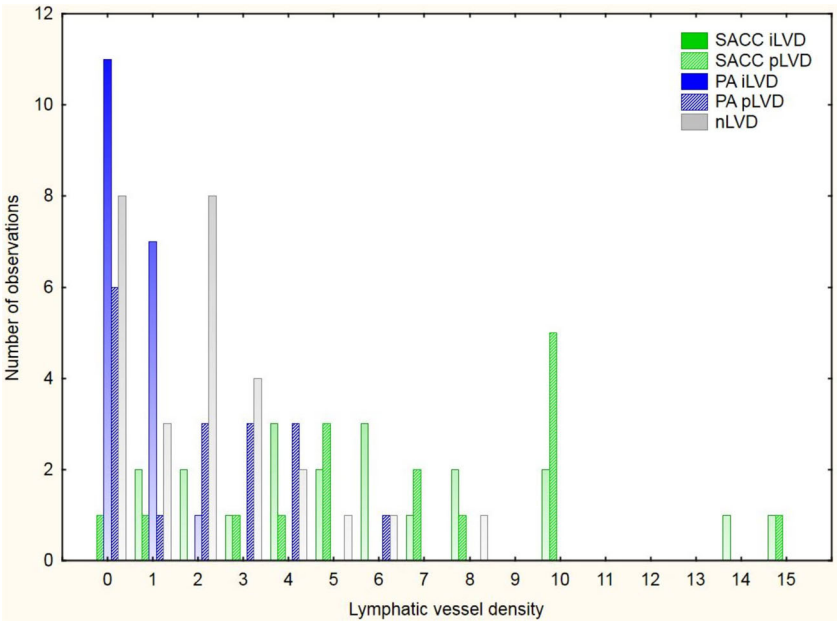

Fig. 2 Comparison of lymphatic vessel densities in salivary adenoid cystic carcinoma and pleomorphic adenoma. Histogram of intratumoral (iLVD), peritumoral (pLVD), and normal salivary parenchyma (nLVD) lymphatic vessel densities in salivary adenoid cystic carcinoma (SACC) and pleomorphic adenoma (PA)

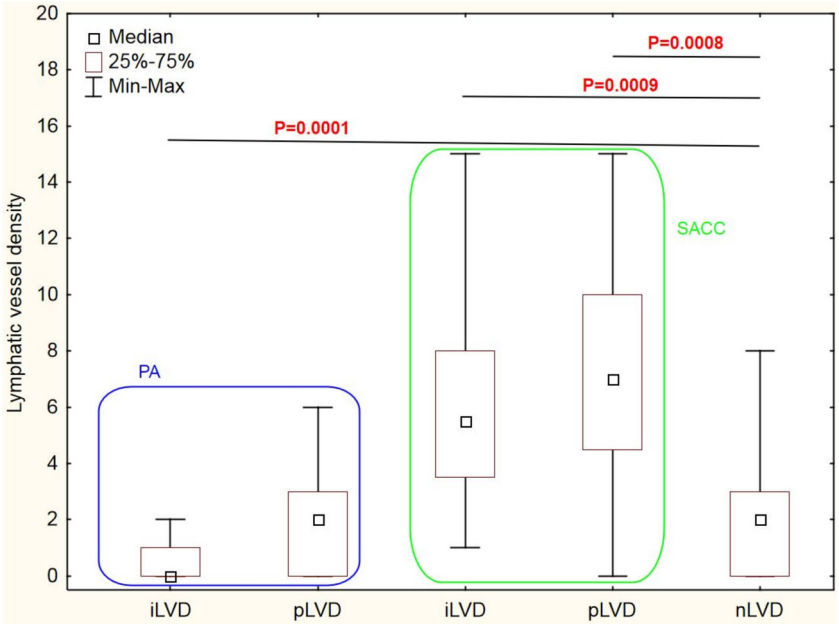

Fig. 3 Comparison of lymphatic vessel densities in salivary adenoid cystic carcinoma and pleomorphic adenoma with healthy salivary parenchyma. *Whiskers boxes graph* showing normal salivary parenchyma (nLVD) lymphatic vessel density with intratumoral (iLVD) and peritumoral (pLVD) lymphatic vessel densities in pleomorphic adenoma (PA) and salivary adenoid cystic carcinoma (SACC)

vessels in Warthin's tumour. The author therefore concluded that tumoral epithelial cells promoted formation of lymphatic capillaries in lymphoid component of this lesion, while those of PA did not. In the present study practically no intratumoral lymphatics were found in PA. The density of peritumoral lymphatics in PA did not exceed that in the normal parenchyma of the surrounding gland. This, along with the results of above cited papers [6, 8, 14] does not support the assumption of lymphangiogenesis in salivary PA.

In another study, Soares [15] examined LVD in 20 minimally and 20 widely invasive carcinomas ex pleomorphic adenoma. In the former group, peritumoral lymphatic vessels markedly outnumbered the intratumoral ones. In the widely invasive tumours, the density of intralymphatic vessel was significantly elevated, equalling to pLVD. The author, therefore, suggested that in the advanced phase of tumour progression, intratumoral lymphatics represented an additional pathway increasing its propensity to metastasize.

Fujita [9] tested a group of 29 SACC for the presence of lymphatic vessels. Solely peritumoral, constricted vessels were detected, with their density not exceeding that of the healthy salivary gland. Consequently, he suggested that lymphangiogenesis does not occur in SACC. On the contrary using identical D2-40 immunostaining, we could demonstrate

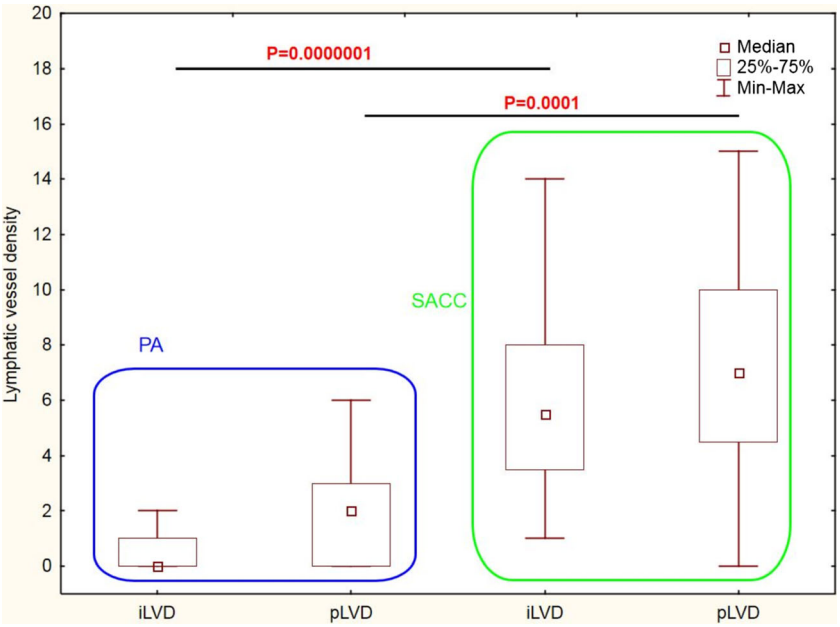

Fig. 4 Comparison of LVD between PA and SACC. Whiskers boxes graph revealing intratumoral (iLVD) and peritumoral (pLVD) lymphatic vessel densities between pleomorphic adenoma (PA) and salivary adenoid cystic carcinoma (SACC)

not only peritumoral but also intratumoral lymphatic vessels in this histopathologic entity. Intratumoral lymphatics were not found in certain tumours [16–18]. However, they were demonstrated in some other malignancies, i.e. in the head and neck squamous cell cancer [19]. Intratumoral lymphatics were also present in broad histopathologic spectrum of 75 salivary carcinomas (including 15 SACC), analysed by Mello [20], with their density being significantly lower than that of peritumoral vessels. The author concluded that lymphangiogenesis occurred in salivary carcinomas. In that study markedly higher density of peritumoral lymphatics was reported. This strongly contrasts with our study, in which no difference between intra- and peritumoral lymphatics in SACC could be demonstrated. Moreover, we found density of both the peritumoral and intratumoral lymphatics in this tumour to outnumber significantly that in normal salivary gland and PA as well. Such differences in distribution and density of lymphatic vessels between benign salivary pleomorphic adenoma and SACC suggest the ability of the latter malignancy to stimulate the formation of new lymphatics, which corresponds to its biologic aggressiveness.

In our study the majority of intratumoral vessels in SACC showed compression, while the peritumoral ones were mostly dilated. This phenomenon, described in other non salivary carcinomas is considered to be due compression of lymphatic channels by neoplastic cells, multiplicating in confined space of tumour core [21]. However, these compressed lymphatics can contribute to the spread of tumoral cells [22].

The authors present the very first study revealing difference in LVD between PA a SACC. Significantly higher values, outnumbering those in healthy salivary parenchyma and PA, along with the presence of relatively numerous intratumoral channels in SACC suggest the capability of this biologically aggressive neoplasm to induce lymphangiogenesis as other salivary carcinomas are believed to do. This conclusion supports the idea of SACC to spread via lymphatic vessels.

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Compliance with Ethical Standards

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Conflict of Interest The authors declare that they have no conflict of interest.

Ethical Approval All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Informed Consent For this type of study formal informed consent is not required.

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