Extracellular Vesicles in Head and Neck Surgery

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EVs has spread from various medical fields to otorhinolaryngology, as well as head and neck surgery (ORL-HNS).

Keywords: extracellular vesicles ; immunomodulation ; drug delivery carriers

1. Introduction

As defined by the International Society for Extracellular Vesicles (ISEV), EVs are particles naturally released from cells, delimited by a lipid bilayer, which cannot replicate since they do not contain a functional nucleus [1].

2. Applicability of EVs in ORL-HNS

2.1. EV-Based Diagnostics

2.1.1. Head and Neck Cancer

HNSCC typically originates in deeper tissues and is usually harder to see or palpate. For that reason, it is often discovered too late, i.e., when it has already progressed locally or metastasised to the cervical lymph nodes. Approximately two-thirds of patients with HNSCC are diagnosed at an advanced stage, and more than half of them experience a recurrence at least once, 90% of them within the first two years [2]. This contributes to the high burden of HNSCC; prompt diagnosis is therefore crucial [3][4][5][6][7][8].

When it comes to diagnostics, some exosomal molecules' unique and consistent expression patterns make them a promising biomarker in some diseases of ORL-HNS. Since EVs are ubiquitous, they can be isolated from tumour liquid biopsy samples or non-invasively collected body fluids, e.g., saliva, plasma, urine. Although using EVs in these settings is becoming increasingly popular in diagnosis, their use is still limited due to the overlapping cellular structure and composition of the tumour and normal cells [9].

Recently, the most often studied potential EV-related biomarkers from different samples, including the above-mentioned diagnostic methods, have been EV levels.

A study by Hoshino et al. (2020) provided a proteomic analysis of EV and particles (EVPs) from 426 human samples, identifying pan-EVP markers, and biomarkers for EVP isolation, cancer detection and cancer type. In addition, a study of EVP proteomes was run to identify universal EVP markers, improve the isolation of human EVPs and offer a resource for early cancer detection with liquid biopsies. Among the exosome markers investigated, HSPA8 (Heat Shock Protein Family A (Hsp70) Member 8), HSP90AB1 (Heat Shock Protein 90 Alpha Family Class B Member 1), CD9, and ALIX (apoptosis-linked gene 2-interacting protein X), isolated from cells, tissues, and most biofluids, were found to be the most prominent and represent the so-called pan-EVP markers [10].

Tumour tissue biopsies and fine-needle aspiration biopsies are the most commonly used diagnostic methods in ORL-HNS. However, due to their invasive nature and inadequate representation of tumoral heterogeneity, liquid biopsies are being explored as an alternative to track the dynamics of the disease. Liquid biopsy uses a non-solid tissue sample, such as blood, saliva, urine or cerebrospinal fluid, for the same purposes as traditional biopsy. In addition, samples are investigated for specific biomarkers related to the disease in question, most often circulating tumour cells (CTC), circulation tumour DNA (ctDNA) or exosomes, which can confirm the diagnosis and enable a further follow-up [11].

Utilisation of EV Levels as Diagnostic Tools

A comparison of salivary (i.e., derived from saliva) and plasma (i.e., derived from plasma) EVs from patients with oral cancer showed that higher levels of salivary medium/large EVs were associated with higher levels of plasma medium/large EVs, the presence of lymph node metastasis, and therefore, higher TNM stage (T referring to primary

tumour size and site, N describing (regional) lymph node involvement and M the presence of distant metastasis) [12]. Additional studies have revealed that salivary EVs in oral cancer are larger, have a more irregular morphology, aggregate more quickly and show a unique infrared signature (i.e., features when analysed with infrared spectroscopy) [13][14][15]. It remains to be investigated why such morphological changes occur, but they confirm the variations in cancer EVs morphology, which might be a consequence of the total increase in the size of secreted EVs or their aggregation after excretion into body fluids. However, the changes in EV shape, size or structure demonstrate the influence of pathophysiological conditions on modifications at the single-EV level.

A study by Theodoraki et al. (2019) compared the total exosomal protein levels, ratios between tumour-derived exosome (TEX) levels and total exosome levels, and specific immune cell-derived exosomes in patients with HNSCC who remained disease-free at two years after therapy, with those whose disease had recurred in the 2-year follow-up. In addition, the study evaluated the value of plasma-derived CD3(+) exosomes as an alternative for T cell isolation and CD3(-) TEX as an alternative for tumour biopsies. Patients with locally advanced HNSCC included in the study received a combination therapy with cetuximab, ipilimumab and radiation therapy. In cases that responded to treatment, ultimately, the total exosomal protein levels, TEX levels/total exosome levels ratios, and levels of total CD3+, CD3(-)PD-L1+ and CD3+15s+ (regulatory T cells) exosomes decreased or remained unchanged compared to pre-treatment. In contrast, their levels increased in patients who had experienced a recurrence [16]. These results support the predictive role of exosomes as non-invasively acquired biomarkers in HNSCC.

Diagnosis Based on DNA Content in EVs

Little research has been done on EV DNA and its relation to HNC and cancer other than HNC. Some studies suggest that EVs may contain tumour-specific DNA [17][18], and others have additionally shown that EVs are loaded with DNA fragments without any apparent selectivity [19]. In relation to HNC, more research is yet to be done on the topic, and should focus on detecting tumour and human papillomavirus (HPV)-specific DNA, which plays a vital role in HNSCC pathogenesis.

Diagnosis Based on RNA Content in EVs

More recently, the ability of EVs to transport different types of RNAs, such as messenger RNAs (mRNAs), micro RNAs (miRNAs or miR), long non-coding RNAs (lncRNAs) and circular RNAs (circRNAs), to target cells has become an essential topic of research. So far, the most frequently studied have been miRNAs, which are small, non-coding RNAs that take part in RNA silencing and the post-transcriptional regulation of gene expression. miRNAs are therefore involved in dysregulations, which lead to the development and invasion of many types of cancer. For that reason, EV-derived miRNAs have been studied as potential biomarkers in different types of tumours, including HNSCC [20]. In a recent systemic review, specific exosomal miRNAs from saliva that showed tremendous potential for use as oral and oropharyngeal cancer biomarkers included miR-10b-5p, miR-486-5p, miR-24-3p and miR-200a. All of these are involved in sustaining a favourable microenvironment for tumour growth, whether through modulation of the immune response, tumour cell cycle and proliferation, growth and migration, or invasion and metastasis [21].

Another critical aspect in HNC diagnosis that has been considered in EV profiling is HPV status. HPV+ oropharyngeal cancer shows different molecular, histopathologic and clinical characteristics, affects a different group of patients, and may require less aggressive therapy, with a better outcome than HPV- cancer [22]. An analysis of exosomes produced by HNSCC showed a distinction between HPV+ and HPV- cancer cells. In terms of their immune response, only HPV- cell-derived exosomes suppressed the maturation of dendritic cells and the expression of proteins involved in antigen processing machinery. Further investigations of exosomal miRNA revealed the overexpression of specific miRNAs, such as miR-1972 in HPV- cells and miR-205-5p in HPV+ cells, which may indicate their role in the alteration of antigen processing machinery and tumour immune responses [23].

Another study, by Ramayanti et al. (2019), performed exosomal miRNA sequencing in patients with nasopharyngeal cancer, which is proven to be associated with EBV (Epstein–Barr virus) infection. The study identified a crucial positive correlation between higher circulating EV BART13-3p miRNA levels and nasopharyngeal cancer compared to healthy individuals or those with asymptomatic EBV infection. miR-BART13-3p promotes the migration of tumour cells and metastasis by driving epithelial–mesenchymal transition via the downregulation of the tumour suppressor AB12 [24]. Compared to healthy individuals, its detection appeared to be most specific and selective for nasopharyngeal cancer status in the early and late stages of the disease. This EV biomarker seems to outperform the more classic EBV-DNA load or EBV IgA serology, since it showed higher sensitivity and specificity than the other two more traditional methods [25].

circRNA consists of large, non-coding RNA, which plays a vital role in gene expression regulation by inhibiting miRNAs. Due to its more excellent stability because of its circular structure, it has been thought to have more potential use as a biomarker than linear RNA. The upregulation of specific types of circRNA has been related to laryngeal cancer [26] and the staging of oral cancer [27].

Diagnosis Based on Protein Content in EVs

Since EVs function as carriers for signal molecules, most of which are proteins, several studies have investigated this type of cargo as a potential biomarker. So far, some of the potential candidates with overexpression in HNSCC-derived EVs are EGFR (epidermal growth factor receptor), PD-L1 (programmed death-ligand 1) and CD44, promoting increased proliferation, migration and metastatic potential in tumoral cells, as well as a poor prognosis [9]. Another example was the isolation of EV proteins from metastatic oral cancer cells, whereby the molecular chaperones HSP90 (heat shock protein 90), TRAP1(TNF receptor associated protein 1) and HSP105 (heat shock protein 105) were shown to be the most promising metastatic and prognostic biomarkers. They play an essential role in protein-folding, stabilising growth factor receptors and regulating anti-apoptotic pathways, with their increased expression coinciding with a worse prognosis [28].

A study by Qu et al. (2021) has identified a set of EV proteins, dysregulated in EVs, isolated from the plasma of patients with oral tongue squamous cell cancer with or without neck lymph node metastasis. Thus, out of many potential candidates, a lot of them have been established as a potential biomarker for oral tongue squamous cell cancer, some of them (such as platelet factor 4 variant, tubulin beta-4A chain, histone H2B type 2-E, and collagen alpha-1) being informative of nodal status as well [29].

2.1.2. Sino-Nasal Diseases

Diagnosis Based on EV miRNA Profile

A recent study compared healthy individuals, analysing EVs and their miRNA profiles in nasal lavage fluid from patients with chronic rhinosinusitis (CRS). The results showed a statistically significant difference in the expression of specific EV miRNAs (five upregulated and seven downregulated) between patients with CRS and healthy subjects. There was also a statistically significant difference in the expression of miRNAs between patients with CRS with nasal polyposis (CRSwNP) and patients with CRS without nasal polyposis (CRSsNP). The altered miRNA expression allegedly promotes the biosynthesis of specific glycans, which are an essential mechanism for CRS development. In addition, other upregulated or downregulated miRNAs act on specific signalling pathways in the nasal mucosa responsible for tissue remodelling in nasal polyps [3].

A different analysis has suggested the essential role of miR-22-3p isolated from nasal lavage fluid exosomes of patients with CRSwNP, whose overexpression increases vascular permeability by targeting endothelial membrane proteins, aggravating inflammation and tissue oedema [30].

Diagnosis Based on EV Protein Content

Researchers have suggested that exosomes derived from the nasal mucosa epithelial cells of patients with CRSwNP are partially responsible for creating conditions for abnormal epithelial growth. At the same time, these exosomes contain CRSwNP-specific proteins, which could potentially serve as biomarkers of the persistence or recurrence of nasal polyps after treatment, and facilitate the development of targeted delivery for antagonistic exosomes or anti-exosomal antibodies to neutralise the effects of endogenous exosomes [4].

Several studies have confirmed the altered protein structure and function, or overexpression, of specific proteins in exosomes isolated from nasal mucosa of patients with CRSwNP and CRSsNP. The overexpression of serine protease inhibitors with the downregulation of the fibrinolysis pathway has also been found. This leads to fibrin accumulation in polypoid tissue, a pro-inflammatory environment, and tissue remodelling with fibrosis [31]. Moreover, integrin β6 upregulation and the upregulation of the biomarkers involved in endothelial to mesenchymal transformation have been found in basal cells of patients with severe CRSwNP [32]. In exosomes isolated from nasal lavage fluid, airway mucin 5AC upregulation, which promotes tissue remodelling and angiogenesis, was found in patients with nasal polyposis [33].

2.1.3. Hearing Disorders

In a study investigating the role of exosomes in middle ear cholesteatoma, keratinocytes and fibroblasts were collected during mastoidectomy and then isolated. The study showed that miRNA-17 from keratinocyte exosomes upregulates fibroblast protein expression, promoting the differentiation of osteoclasts and, therefore, bone destruction, and is the essential factor in cholesteatoma pathogenesis [7].

Another study examined the protective role of exosomes in the inner ear. Exosomes isolated from mice utricle, especially supporting cells, exhibited a specific surface-associated protein, HSP70 (70 kilodalton heat shock protein), which interacts with sensory cell surface protein TLR4 (toll-like receptor 4) and further promotes its survival. The exact intracellular signalling pathway of promotion has not been well studied so far. However, since it has been proven that exosomes have an essential role in intracellular communication, protection and promotion of survival in the inner ear, they make a good potential therapeutic deliverer to the site [5].

A new potential use of exosomes has been discovered in terms of their neuroprotective abilities and hearing loss protection in the inner ear. A first in vivo study used human UC-MSC (umbilical cord-derived mesenchymal stem cells) EVs to investigate their ability to prevent auditory neuronal damage after exposure to noise trauma. Their use appears to be beneficial, with support of neuronal survival and noise-induced damage repair, presumably by a combination of the EVs' effects on immunomodulatory cytokines, the modification of the intracellular transduction of hair cell function, the morphology of auditory neurons and the release of EV miRNA, which further regulates signalling in target cells, primarily to alter their immune response [6].

Similarly, another study investigated the potential of exosomes isolated from mouse inner ear stem cells and their effect on gentamicin-induced ototoxicity. The study showed that isolated miRNA-182-5p could inhibit pro-apoptotic factors and their signalling pathways, which are deregulated during gentamicin-induced hair cell apoptosis. Again, this discovery provides some new therapeutic options, but has yet to be studied further [34].

2.2. EV-Based Therapy

When delivering therapeutics to the site of action, the main obstacles are low accumulation and bioavailability in the target tissue, fast clearance, and the off-target toxicity of the therapeutic. Out of all nanoparticles available, liposomes have been proven to be the most successful delivery vehicles. In terms of their similar properties, EVs also make a probable nano therapy candidate [35]. However, their clinical application remains a challenge. To overcome this challenge, several platforms have been designed to isolate and harvest EVs. These platforms are divided based on their origin: native EVs, EVs from genetically engineered cells, post-modified EVs (drug-loaded or surface modified), and EV-inspired liposomes [36].

2.2.1. Regenerative Therapy

To date, a considerable amount of research has been done on the therapeutic use of platelet- and extracellular vesicle-rich plasma (PVRP). PVRP contains a high concentration of platelets and platelet-derived EVs that are primarily known for their regenerative effect. It has been used for years in various surgical fields. In ORL-HNS, PVRP has been chiefly applied for better post-operative wound healing and pain reduction, usually in a gel [37]. The procedures include chronic temporal bone inflammation treatment [38], repair of auricle trauma [39] and eardrum perforation, posterior external ear canal wall reconstruction [40], mastoid reconstruction [41], rhinoplasty [42] in post-surgical nasal packing [43], frontal sinus obliteration [44], anterior skull base CSF leakage [45], aesthetic procedures on the skin of the face and neck [46], cleft lip [47], lesions of the oral mucosa [48], osteoradionecrosis of the mandible [49], pharyngoplasty in obstructive sleep apnea syndrome [50], esophagocutaneous [51] and pharyngocutaneous fistula [52], and suprafacial parotidectomy [37][53].

In contrast to PVRP, EVs have been used in regenerative medicine as isolates in some non-ORL-HNS preclinical settings, e.g., a study by Otahal et al. (2020) investigating the chondroprotective effects of EVs from autologous blood-derived products in the treatment of osteoarthritis. The characterisation of EVs from CPRP (citrate-anticoagulated platelet-rich plasma) revealed that EVs from enriched blood products are sufficient to cause changes in chondroprotective and chondrogenic gene expression and the modulation of pro-inflammatory signalling on mRNA and protein levels in osteoarthritis chondrocytes, surpassing total blood products[54].

2.2.2. Head and Neck Cancer Treatment

In HNC, EVs have strong potential use as targets of treatment, and also as treatment substances. EVs can be targeted at their synthesis, secretion and internalisation. EVs' unique properties enable them to encapsulate drugs or other bioactive molecules and, therefore, function as drug delivery systems. Moreover, they can directly modulate the immune response in carcinogenesis via the bioactive substances they carry from the mother cell[9].

Treatment via Modulation of EV Synthesis, Secretion and Uptake

There has been much research on this topic since EVs' biosynthesis and release mechanisms could be modulated, which could be applied to treat various diseases.

It was shown by Zhang et al. (2019) that macrophages stimulated with ODN (CpG oligodeoxynucleotides), a TLR9 (Toll-like receptor) agonist, release EVs carrying ODN and Cdc42. These EVs synergistically stimulate naïve macrophages to propagate an intracellular immune response. This study offers a possible efficient approach to treat various inflammatory diseases based on the modulation of EV secretion and uptake[55].

In investigations of the genetic manipulation of exosome secretion in tumoral cells, the RAB family have been proved to regulate exosome secretion[56]. More specifically, Rab27A/B proteins regulate cancer cell-intrinsic properties while also being involved in exchanging exosomes between different cells within the tumour microenvironment in HNSCC. The genetic deletion of both Rab27A and Rab27B in HNSCC cells reduced the exosome-mediated induction of innervation in vitro and in vivo[57].

A study by Madeo et al. (2018) showed that GW4867, a neutral sphingomyelinase inhibitor, reduces the release of CD9+/CD81+ EVs from HNC, which further reduces the infiltration of nerves into tumoral tissue and therefore the progression of the disease[58]. Targeting EV release or blocking the ability of neurons to spread further in the tumour is thus a good focus in HNSCC treatment. However, it is known that EVs are not involved only in tumorigenesis and disease progression, but also in tumour suppression and immune cell activation[59].

Treatment via Inhibition of EV Internalisation

There are many different pathways of EV endocytosis, and they usually depend on proteins expressed on the surface of EV and the target cell. It is therefore likely that EVs are taken up by more than one route. There are several substances known to block EV internalisation, but there is still little evidence of the effects of this inhibition. There is also a lack of knowledge of specific steps in this process in relation to HNC. So far, it has been reported that chemotherapeutic cetuximab could have blocking effects on EVs derived from oral tongue cancer, which may be related to surface EGFR[60].

Treatment via Modulation of EV Cargo Components

With the ability of EVs to transfer biomolecules, they make good drug delivery carrier candidates. There are several known methods of loading EVs with cargo, such as direct incubation with EVs, passive loading by incubation with donor cells, electroporation, sonication, the freeze/thaw method, extrusion, chemical conjugation, and the creation of nanoparticle drug complexes [61]. The goal is to use EVs as carriers and provide better bioavailability and stability to therapeutic molecules [9]. For example, one of the first studies in this field showed that EVs rich with miR-185, derived from mesenchymal stem cells, modulate inflammation, inhibit cell proliferation and angiogenesis, promote apoptosis, and thus inhibit the progression of disease in mice with potential oral malignant disorders[62].

Treatment via Immunomodulation Induced by EVs

The cancer immune cycle includes presenting antigens to antigen presentation cells (APCs) and activating effector T-cells by APCs. Effector T-cells then infiltrate the tumour, where cytotoxic T-cells recognise and kill cancer cells. There are several stimulatory and inhibitory factors included in this pathway, which provide a potential therapeutic target. One of the critical pathways involved in the cancer-immunity cycle, called STING (stimulator of interferon genes), stimulates the interferon genes required to present cancer antigens to T-cells. Recently, a STING agonist combined with the prostaglandin F receptor negative regulator, which activates APCs, has been developed, loaded into EVs, and delivered intratumorally[63]. It was proven that the STING pathway plays an essential role in HPV-related carcinogenesis in HNSCC, which makes it a promising therapeutic for HNC, especially when combined with other already established immunotherapeutics (e.g., cetuximab)[64].

References

1. Théry, C.; Witwer, K.W.; Aikawa, E.; Alcaraz, M.J.; Anderson, J.D.; Andriantsitohaina, R.; Antoniou, A.; Arab, T.; Archer, F.; Atkin-Smith, G.K.; et al. Minimal Information for Studies of Extracellular Vesicles 2018 (MISEV2018): A Position Statement of the International Society for Extracellular Vesicles and Update of the MISEV2014 Guidelines. J. Extracell. Vesicles 2018, 7, 1535750.
2. Gillison, M.L.; D'Souza, G.; Westra, W.; Sugar, E.; Xiao, W.; Begum, S.; Viscidi, R. Distinct Risk Factor Profiles for Human Papillomavirus Type 16—Positive and Human Papillomavirus Type 16—Negative Head and Neck Cancers. JNCI J. Natl. Cancer Inst. 2008, 100, 407–420.
3. Cha, S.; Seo, E.-H.; Lee, S.H.; Kim, K.S.; Oh, C.-S.; Moon, J.-S.; Kim, J.K. MicroRNA Expression in Extracellular Vesicles from Nasal Lavage Fluid in Chronic Rhinosinusitis. Biomedicines 2021, 9, 471.

4. Zhou, M.; Tan, K.S.; Guan, W.; Jiang, L.; Deng, J.; Gao, W.; Lee, Y.M.; Xu, Z.; Luo, X.; Liu, C.; et al. Proteomics Profiling of Epithelium-Derived Exosomes from Nasal Polyps Revealed Signaling Functions Affecting Cellular Proliferation. Respir. Med. 2020, 162, 105871.
5. Breglio, A.M.; May, L.A.; Barzik, M.; Welsh, N.C.; Francis, S.P.; Costain, T.Q.; Wang, L.; Anderson, D.E.; Petralia, R.S.; Wang, Y.-X.; et al. Exosomes Mediate Sensory Hair Cell Protection in the Inner Ear. J. Clin. Investig. 2020, 130, 2657–2672.
6. Warnecke, A.; Harre, J.; Staecker, H.; Prenzler, N.; Strunk, D.; Couillard-Despres, S.; Romanelli, P.; Hollerweger, J.; Lassacher, T.; Auer, D.; et al. Extracellular Vesicles from Human Multipotent Stromal Cells Protect against Hearing Loss after Noise Trauma in Vivo. Clin. Transl. Med. 2020, 10, e262.
7. Gong, N.Y.; Zhu, W.; Xu, R.; Teng, Z.; Deng, C.; Zhou, H.; Xia, M.; Zhao, M. Keratinocytes-Derived Exosomal MiRNA Regulates Osteoclast Differentiation in Middle Ear Cholesteatoma. Biochem. Biophys. Res. Commun. 2020, 525, 341–347.
8. Zhang, Y.; Shi, S.; Xu, Q.; Zhang, Q.; Shanti, R.M.; Le, A.D. SIS-ECM Laden with GMSC-Derived Exosomes Promote Taste Bud Regeneration. J. Dent. Res. 2019, 98, 225–233.
9. Qu, X.; Li, J.-W.; Chan, J.; Meehan, K. Extracellular Vesicles in Head and Neck Cancer: A Potential New Trend in Diagnosis, Prognosis, and Treatment. Int. J. Mol. Sci. 2020, 21, 8260.
10. Hoshino, A.; Kim, H.S.; Bojmar, L.; Gyan, K.E.; Cioffi, M.; Hernandez, J.; Zambirinis, C.P.; Rodrigues, G.; Molina, H.; Heissel, S.; et al. Extracellular Vesicle and Particle Biomarkers Define Multiple Human Cancers. Cell 2020, 182, 1044–1061.e18.
11. Burklund, A.; Tadimety, A.; Nie, Y.; Hao, N.; Zhang, J.X.J. Advances in Diagnostic Microfluidics. Adv. Clin. Chem. 2020, 95, 1–72.
12. Zhong, W.; Ren, J.; Xiong, X.; Man, Q.; Zhang, W.; Gao, L.; Li, C.; Liu, B.; Sun, Z.; Jia, J.; et al. Increased Salivary Microvesicles Are Associated with the Prognosis of Patients with Oral Squamous Cell Carcinoma. J. Cell. Mol. Med. 2019, 23, 4054–4062.
13. Sharma, S.; Gillespie, B.M.; Palanisamy, V.; Gimzewski, J.K. Quantitative Nanostructural and Single-Molecule Force Spectroscopy Biomolecular Analysis of Human-Saliva-Derived Exosomes. Langmuir 2011, 27, 14394–14400.
14. Zlotogorski-Hurvitz, A.; Dekel, B.Z.; Malonek, D.; Yahalom, R.; Vered, M. FTIR-Based Spectrum of Salivary Exosomes Coupled with Computational-Aided Discriminating Analysis in the Diagnosis of Oral Cancer. J. Cancer Res. Clin. Oncol. 2019, 145, 685–694.
15. Zlotogorski-Hurvitz, A.; Dayan, D.; Chaushu, G.; Salo, T.; Vered, M. Morphological and Molecular Features of Oral Fluid-Derived Exosomes: Oral Cancer Patients versus Healthy Individuals. J. Cancer Res. Clin. Oncol. 2016, 142, 101–110.
16. Theodoraki, M.-N.; Yerneni, S.; Gooding, W.E.; Ohr, J.; Clump, D.A.; Bauman, J.E.; Ferris, R.L.; Whiteside, T.L. Circulating Exosomes Measure Responses to Therapy in Head and Neck Cancer Patients Treated with Cetuximab, Ipilimumab, and IMRT. OncoImmunology 2019, 8, e1593805.
17. Vagner, T.; Spinelli, C.; Minciacchi, V.R.; Balaj, L.; Zandian, M.; Conley, A.; Zijlstra, A.; Freeman, M.R.; Demichelis, F.; De, S.; et al. Large Extracellular Vesicles Carry Most of the Tumour DNA Circulating in Prostate Cancer Patient Plasma. J. Extracell. Vesicles 2018, 7, 1505403.
18. Kahlert, C.; Melo, S.A.; Protopopov, A.; Tang, J.; Seth, S.; Koch, M.; Zhang, J.; Weitz, J.; Chin, L.; Futreal, A.; et al. Identification of Double-Stranded Genomic DNA Spanning All Chromosomes with Mutated KRAS and P53 DNA in the Serum Exosomes of Patients with Pancreatic Cancer. J. Biol. Chem. 2014, 289, 3869–3875.
19. Jeppesen, D.K.; Fenix, A.M.; Franklin, J.L.; Higginbotham, J.N.; Zhang, Q.; Zimmerman, L.J.; Liebler, D.C.; Ping, J.; Liu, Q.; Evans, R.; et al. Reassessment of Exosome Composition. Cell 2019, 177, 428–445.e18.
20. Min, L.; Zhu, S.; Chen, L.; Liu, X.; Wei, R.; Zhao, L.; Yang, Y.; Zhang, Z.; Kong, G.; Li, P.; et al. Evaluation of Circulating Small Extracellular Vesicles Derived MiRNAs as Biomarkers of Early Colon Cancer: A Comparison with Plasma Total MiRNAs. J. Extracell. Vesicles 2019, 8, 1643670.
21. Faur, C.I.; Rotaru, H.; Osan, C.; Jurj, A.; Roman, R.C.; Moldovan, M.; Chirila, M.; Hedesiu, M. Salivary Exosomal MicroRNAs as Biomarkers for Head and Neck Cancer Detection-a Literature Review. Maxillofac. Plast. Reconstr. Surg. 2021, 43, 19.
22. Leemans, C.R.; Braakhuis, B.J.M.; Brakenhoff, R.H. The Molecular Biology of Head and Neck Cancer. Nat. Rev. Cancer 2011, 11, 9–22.
23. Ludwig, S.; Sharma, P.; Wise, P.; Sposto, R.; Hollingshead, D.; Lamb, J.; Lang, S.; Fabbri, M.; Whiteside, T.L. MRNA and MiRNA Profiles of Exosomes from Cultured Tumor Cells Reveal Biomarkers Specific for HPV16-Positive and

HPV16-Negative Head and Neck Cancer. Int. J. Mol. Sci. 2020, 21, 8570.

24. Huang, J.; Qin, Y.; Yang, C.; Wan, C.; Dai, X.; Sun, Y.; Meng, J.; Lu, Y.; Li, Y.; Zhang, Z.; et al. Downregulation of ABI2 Expression by EBV-MiR-BART13-3p Induces Epithelial-Mesenchymal Transition of Nasopharyngeal Carcinoma Cells through Upregulation of c-JUN/SLUG Signaling. Aging 2020, 12, 340–358.
25. Ramayanti, O.; Verkuijlen, S.A.W.M.; Novianti, P.; Scheepbouwer, C.; Misovic, B.; Koppers-Lalic, D.; Weering, J.; Beckers, L.; Adham, M.; Martorelli, D.; et al. Vesicle-bound EBV-BART13-3p MiRNA in Circulation Distinguishes Nasopharyngeal from Other Head and Neck Cancer and Asymptomatic EBV-infections. Int. J. Cancer 2019, 144, 2555–2566.
26. Tian, L.; Cao, J.; Jiao, H.; Zhang, J.; Ren, X.; Liu, X.; Liu, M.; Sun, Y. CircRASSF2 Promotes Laryngeal Squamous Cell Carcinoma Progression by Regulating the MiR-302b-3p/IGF-1R Axis. Clin. Sci. 2019, 133, 1053–1066.
27. Luo, Y.; Liu, F.; Guo, J.; Gui, R. Upregulation of Circ_0000199 in Circulating Exosomes Is Associated with Survival Outcome in OSCC. Sci. Rep. 2020, 10, 13739.
28. Ono, K.; Eguchi, T.; Sogawa, C.; Calderwood, S.K.; Futagawa, J.; Kasai, T.; Seno, M.; Okamoto, K.; Sasaki, A.; Kozaki, K. HSP-enriched Properties of Extracellular Vesicles Involve Survival of Metastatic Oral Cancer Cells. J. Cell. Biochem. 2018, 119, 7350–7362.
29. Qu, X.; Leung, T.C.N.; Ngai, S.-M.; Tsai, S.-N.; Thakur, A.; Li, W.-K.; Lee, Y.; Leung, L.; Ng, T.-H.; Yam, J.; et al. Proteomic Analysis of Circulating Extracellular Vesicles Identifies Potential Biomarkers for Lymph Node Metastasis in Oral Tongue Squamous Cell Carcinoma. Cells 2021, 10, 2179.
30. Zhang, W.; Zhang, T.; Yan, Y.; Zhang, J.; Zhou, Y.; Pei, Y.; Yao, L.; You, B.; Chen, J. Exosomal MiR-22-3p Derived from Chronic Rhinosinusitis with Nasal Polyps Regulates Vascular Permeability by Targeting VE-Cadherin. BioMed Res. Int. 2020, 2020, 1237678.
31. Mueller, S.K.; Nocera, A.L.; Dillon, S.T.; Libermann, T.A.; Wendler, O.; Bleier, B.S. Tissue and Exosomal Serine Protease Inhibitors Are Significantly Overexpressed in Chronic Rhinosinusitis With Nasal Polyps. Am. J. Rhinol. Allergy 2019, 33, 359–368.
32. Takahashi, T.; Kato, A.; Suh, L.A.; Carter, R.G.; Stevens, W.W.; Price, C.P.E.; Norton, J.E.; Weibman, A.R.; Harris, K.E.; Peters, A.T.; et al. Integrin B6 Microparticles in Nasal Lavage Fluids; Potential New Biomarkers for Basal Cell Activation in Chronic Rhinosinusitis. Allergy 2020, 75, 3261–3264.
33. Wang, L.-F.; Lee, C.-H.; Liang, S.-S.; Hung, C.-C.; Wu, Y.-R.; Chien, C.-Y.; Lee, C.-H.; Chen, J.Y.-F. Mucin 5AC Is Significantly Upregulated in Exosomes from the Nasal Lavage Fluid and May Promote the Expression of COX-2, VEGF and MMP-9: An Implication in Nasal Polyp Pathogenesis. Rhinol. J. 2021, 59, 328–336.
34. Lai, R.; Cai, C.; Wu, W.; Hu, P.; Wang, Q. Exosomes Derived from Mouse Inner Ear Stem Cells Attenuate Gentamicin-induced Ototoxicity in Vitro through the MiR-182-5p/FOXO3 Axis. J. Tissue Eng. Regen. Med. 2020, 14, 1149–1156.
35. de Castilla, P.E.M.; Tong, L.; Huang, C.; Sofias, A.M.; Pastorin, G.; Chen, X.; Storm, G.; Schiffelers, R.M.; Wang, J.-W. Extracellular Vesicles as a Drug Delivery System: A Systematic Review of Preclinical Studies. Adv. Drug Deliv. Rev. 2021, 175, 113801.
36. Herrmann, I.K.; Wood, M.J.A.; Fuhrmann, G. Extracellular Vesicles as a Next-Generation Drug Delivery Platform. Nat. Nanotechnol. 2021, 16, 748–759.
37. Vozel, D.; Božič, D.; Jeran, M.; Jan, Z.; Pajnič, M.; Pađen, L.; Uršič, B.; Iglič, A.; Kralj-Iglič, V.; Battelino, S. Treatment with Platelet- and Extracellular Vesicle-Rich Plasma in Otorhinolaryngology-a Review and Future Perspectives. In Advances in Biomembranes and Lipid Self-Assembly; Kruze: Oxford, UK, 2021; pp. 119–153.
38. Vozel, D.; Božič, D.; Jeran, M.; Jan, Z.; Pajnič, M.; Pađen, L.; Steiner, N.; Kralj-Iglič, V.; Battelino, S. Autologous Platelet- and Extracellular Vesicle-Rich Plasma Is an Effective Treatment Modality for Chronic Postoperative Temporal Bone Cavity Inflammation: Randomized Controlled Clinical Trial. Front. Bioeng. Biotechnol. 2021, 9, 677541.
39. Lee, S.K.; Lim, Y.M.; Lew, D.H.; Song, S.Y. Salvage of Unilateral Complete Ear Amputation with Continuous Local Hyperbaric Oxygen, Platelet-Rich Plasma and Polydeoxyribonucleotide without Micro-Revascularization. Arch. Plast. Surg. 2017, 44, 554–558.
40. Elbary, M.; Nasr, W.; Sorour, S. Platelet-Rich Plasma in Reconstruction of Posterior Meatal Wall after Canal Wall Down Mastoidectomy. Int. Arch. Otorhinolaryngol. 2018, 22, 103–107.
41. Askar, S.M.; Saber, I.M.; Omar, M. Mastoid Reconstruction With Platelet-Rich Plasma and Bone Pate After Canal Wall Down Mastoidectomy: A Preliminary Report. Ear Nose Throat J. 2021, 100, 485–489.
42. Gode, S.; Ozturk, A.; Kısmalı, E.; Berber, V.; Turhal, G. The Effect of Platelet-Rich Fibrin on Nasal Skin Thickness in Rhinoplasty. Facial Plast. Surg. 2019, 35, 400–403.

43. Kuzucu, I.; Beriat, G.K.; Ezerarslan, H.; Ozdemir, S.; Kocaturk, S. Effects of the Autologous Platelet-Rich Plasma in Nasal Pack on Postoperative Quality of Life. J. Craniofacial Surg. 2017, 28, e299–e302.

44. Mendonça-Caridad, J.J.; Juiz-Lopez, P.; Rubio-Rodriguez, J.P. Frontal Sinus Obliteration and Craniofacial Reconstruction with Platelet Rich Plasma in a Patient with Fibrous Dysplasia. Int. J. Oral Maxillofac. Surg. 2006, 35, 88–91.

45. Khafagy, Y.W.; Elfattah, A.M.A.; Moneir, W.; Salem, E.H. Leukocyte- and Platelet-Rich Fibrin: A New Graft Material in Endoscopic Repair of Spontaneous CSF Leaks. Eur. Arch. Oto-Rhino-Laryngol. 2018, 275, 2245–2252.

46. Maisel-Campbell, A.L.; Ismail, A.; Reynolds, K.A.; Poon, E.; Serrano, L.; Grushchak, S.; Farid, C.; West, D.P.; Alam, M. A Systematic Review of the Safety and Effectiveness of Platelet-Rich Plasma (PRP) for Skin Aging. Arch. Dermatol. Res. 2020, 312, 301–315.

47. Refahee, S.M.; Aboulhassan, M.A.; Aziz, O.A.; Emara, D.; Seif El Dein, H.M.; Moussa, B.G.; Abu Sneineh, M. Is PRP Effective in Reducing the Scar Width of Primary Cleft Lip Repair? A Randomized Controlled Clinical Study. Cleft Palate-Craniofacial J. 2020, 57, 581–588.

48. Mahajan, M.; Gupta, M.K.; Bande, C.; Meshram, V. Comparative Evaluation of Healing Pattern After Surgical Excision of Oral Mucosal Lesions by Using Platelet-Rich Fibrin (PRF) Membrane and Collagen Membrane as Grafting Materials —A Randomized Clinical Trial. J. Oral Maxillofac. Surg. 2018, 76, 1469.e1–1469.e9.

49. Scala, M.; Gipponi, M.; Mereu, P.; Strada, P.; Corvò, R.; Muraglia, A.; Massa, M.; Bertoglio, S.; Santi, P.; Cafiero, F. Regeneration of Mandibular Osteoradionecrosis Defect with Platelet Rich Plasma Gel. In Vivo 2010, 24, 889–893.

50. Elkahwagi, M.; Elokda, M.; Elghannam, D.; Elsobki, A. Role of Autologous Platelet-Rich Fibrin in Relocation Pharyngoplasty for Obstructive Sleep Apnoea. Int. J. Oral Maxillofac. Surg. 2020, 49, 200–206.

51. Joudi, M.; Hamidi Alamdari, D.; Hyradfar, M.; Rahimi, H.R.; Saremi, E.; Fathi, M.; Shojaeian, R.; Koliakos, G. Lateral Traumatic Esophago-Cutaneous Fistula in a Child; Platelet-Rich Fibrin Glue Challenge. Iran. Red Crescent Med J. 2013, 15, 256–259.

52. Eryılmaz, A.; Demirci, B.; Gunel, C.; Kacar Doger, F.; Yukselen, O.; Kurt Omurlu, I.; Basal, Y.; Agdas, F.; Basak, S. Can Tissue Adhesives and Platelet-Rich Plasma Prevent Pharyngocutaneous Fistula Formation? Auris Nasus Larynx 2016, 43, 62–67.

53. Ricci, E.; Riva, G.; Dagna, F.; Cavalot, A.L. The Use of Platelet-Rich Plasma Gel in Superficial Parotidectomy. Acta Otorhinolaryngol. Ital. 2019, 39, 363–366.

54. Otahal, A.; Kramer, K.; Kuten-Pella, O.; Weiss, R.; Stotter, C.; Lacza, Z.; Weber, V.; Nehrer, S.; de Luna, A. Characterization and Chondroprotective Effects of Extracellular Vesicles From Plasma- and Serum-Based Autologous Blood-Derived Products for Osteoarthritis Therapy. Front. Bioeng. Biotechnol. 2020, 8, 584050.

55. Zhang, Y.; Jin, X.; Liang, J.; Guo, Y.; Sun, G.; Zeng, X.; Yin, H. Extracellular Vesicles Derived from ODN-Stimulated Macrophages Transfer and Activate Cdc42 in Recipient Cells and Thereby Increase Cellular Permissiveness to EV Uptake. Sci. Adv. 2019, 5, eaav1564.

56. Banks, W.A.; Sharma, P.; Bullock, K.M.; Hansen, K.M.; Ludwig, N.; Whiteside, T.L. Transport of Extracellular Vesicles across the Blood-Brain Barrier: Brain Pharmacokinetics and Effects of Inflammation. Int. J. Mol. Sci. 2020, 21, 4407.

57. McAndrews, K.M.; Kalluri, R. Mechanisms Associated with Biogenesis of Exosomes in Cancer. Mol. Cancer 2019, 18, 52.

58. Madeo, M.; Colbert, P.L.; Vermeer, D.W.; Lucido, C.T.; Cain, J.T.; Vichaya, E.G.; Grossberg, A.J.; Muirhead, D.; Rickel, A.P.; Hong, Z.; et al. Cancer Exosomes Induce Tumor Innervation. Nat. Commun. 2018, 9, 4284.

59. Whiteside, T.L. Exosomes and Tumor-Mediated Immune Suppression. J. Clin. Investig. 2016, 126, 1216–1223.

60. Licitra, L.; Mesia, R.; Rivera, F.; Remenár, É.; Hitt, R.; Erfán, J.; Rottey, S.; Kawecki, A.; Zabolotnyy, D.; Benasso, M.; et al. Evaluation of EGFR Gene Copy Number as a Predictive Biomarker for the Efficacy of Cetuximab in Combination with Chemotherapy in the First-Line Treatment of Recurrent and/or Metastatic Squamous Cell Carcinoma of the Head and Neck: EXTREME Study. Ann. Oncol. Off. J. Eur. Soc. Med. Oncol. 2011, 22, 1078–1087.

61. Elsharkasy, O.M.; Nordin, J.Z.; Hagey, D.W.; de Jong, O.G.; Schiffelers, R.M.; Andaloussi, S.E.; Vader, P. Extracellular Vesicles as Drug Delivery Systems: Why and How? Adv. Drug Deliv. Rev. 2020, 159, 332–343.

62. Wang, L.; Yin, P.; Wang, J.; Wang, Y.; Sun, Z.; Zhou, Y.; Guan, X. Delivery of Mesenchymal Stem Cells-Derived Extracellular Vesicles with Enriched MiR-185 Inhibits Progression of OPMD. Artif. Cells Nanomed. Biotechnol. 2019, 47, 2481–2491.

63. Jang, S.C.; Moniz, R.J.; Sia, C.L.; Harrison, R.A.; Houde, D.; Ross, N.; Xu, K.; Lewis, N.; Bourdeau, R.; McCoy, C.; et al. Abstract 944: ExoSTING: An Engineered Exosome Therapeutic That Selectively Delivers STING Agonist to the

Tumor Resident Antigen-Presenting Cells Resulting in Improved Tumor Antigen-Specific Adaptive Immune Response. In Proceedings of the AACR Annual Meeting 2019, Atlanta, GA, USA, 29 March–3 April 2019; American Association for Cancer Research: Philadelphia, PA, USA, 2019.

64. Lu, S.; Concha-Benavente, F.; Shayan, G.; Srivastava, R.M.; Gibson, S.P.; Wang, L.; Gooding, W.E.; Ferris, R.L. STING Activation Enhances Cetuximab-Mediated NK Cell Activation and DC Maturation and Correlates with HPV+ Status in Head and Neck Cancer. Oral Oncol. 2018, 78, 186–193.