

Calcitonin gene-related peptide: A promising bridge between cancer development and cancer-associated pain in oral squamous cell carcinoma (Review)

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Abstract. Nerves have been widely demonstrated to exert major effects in tumor-associated microenvironments. Due to the characteristic innervation of the oral cavity and the fact that cancer-associated pain is a distinct feature of oral squamous cell carcinoma (OSCC), the sensory nerves may dominate in the OSCC-nerve microenvironment. As the most abundant neuropeptide in the trigeminal ganglion, the calcitonin gene-related peptide (CGRP) exerts a dual effect on cancer development and cancer-associated pain in various types of cancer. The present review explored the potential molecular mechanisms of the roles of CGRP in cancer development and cancer-associated pain, suggesting that CGRP may be a promising therapeutic target for OSCC.

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1. Introduction

Similarly to normal organs, tumors establish their own microenvironment to fulfill their dynamic growing demands. This microenvironment may include the extracellular matrix, vessels, lymphangions and, as an increasingly recognized compartment, nerves (1). The role of vessels in tumor microenvironments has been widely explored (2,3). However, so far the curative effects of anti-angiogenesis treatments have been proven dissatisfying, and more and more studies find that the nerves play a pivotal role in various cancer (4,5).

Oral cancer is a common malignant cancer type in the head and neck region with over 50,000 new cases and 10,000 new deaths every year in the U.S.A (6). Oral squamous cell carcinoma (OSCC) constitutes 90% of cases of oral cancer (6). OSCC is associated with severe disease and treatment-associated morbidity, and is frequently reported as having high rates of recurrence despite advances in cancer treatment (7). The oral cavity is innervated by cranial nerves with a high density of sensory nerves, particularly the trigeminal nerve (8). Therefore, pain is the most frequent complaint at the primary site (9), and the prevalence and intensity of OSCC-associated pain are higher than those in all other types of cancer (10,11). The mechanisms involved in the OSCC-nerve microenvironment are likely to differ from those of prostate, pancreatic and breast cancer, which are predominantly innervated by autonomic nerves (4,12).

As the most abundant neuropeptide in the trigeminal ganglion, the calcitonin gene-related peptide (CGRP) exerts a dual effect on both cancer development and cancer-associated pain in various types of cancer, such as osteosarcoma (13) and breast cancer (14). The present review discussed the potential molecular mechanisms by which CGRP is implicated in the development of OSCC and associated pain, suggesting

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Abbreviations: OSCC, oral squamous cell carcinoma; CGRP, calcitonin gene-related peptide; TG, trigeminal ganglion; CLR, calcitonin receptor-like receptor; RAMP1, receptor activity-modifying protein 1; RCP, receptor component protein; GPCR, G-protein-coupled receptor; DRG, dorsal root ganglion; AMPK, adenosine monophosphate-activated protein kinase

Key words: oral squamous cell carcinoma, calcitonin gene-related peptide, cancer-associated pain, metabolic reprogramming, internalization, immune evasion

that CGRP may be a promising therapeutic target in OSCC (Fig. 1).

2. CGRP and its inhibitors

CGRP is a 37-amino acid peptide that has a ubiquitous distribution, particularly in trigeminal ganglions (TGs) (15). CGRP has two major isoforms: α - and β -CGRP, which have similar structures and biological activities, but are encoded by separate genes (*CALCA* for α -CGRP and *CALCB* for β -CGRP) (16). In general, it is thought that α -CGRP is the cardinal form occurring in the central and peripheral nervous system, whereas β -CGRP, the only product of the *CALCB* gene, acts mainly in the enteric nervous system (16). Along with the wide innervation of C and A δ fibers, CGRP exerts various physical effects, including the regulation of energy metabolism, cardiovascular function and pain conduction (17). CGRP has crucial functions in pathological situations, including hypertension, migraine, atherosclerosis and vessel remodeling, which has been elaborated on in a previous review (17).

The CGRP receptor is present in both central and peripheral nerves (18). It is composed of three proteins, namely the calcitonin receptor-like receptor (CLR), receptor activity-modifying protein 1 (RAMP1) and receptor component protein (RCP) (18). The CLR belongs to the class B 'secretin-like' family of G-protein-coupled receptors (GPCRs) (18). RAMP1s are small membrane proteins that possess a large extracellular NH₂ terminus of 100 amino acids, a single transmembrane domain and a short intracellular domain of 10 amino acids (19). RCP is a 16-kDa protein that interacts with the second intracellular cytoplasmic loop of the CLR (20). It is hypothesized that the CLR, RAMP1 and RCP compose a high-affinity receptor complex for CGRP that may be antagonized by the short peptide CGRP₈₋₃₇ and non-peptide antagonists, such as rimegepant (21).

With the revelation of the pivotal role of the CGRP/CLR axis in migraines (22), successful therapeutic strategies using humanized monoclonal antibodies or non-peptide antagonists directed against CGRP or CLR have emerged. Antibodies against CGRP, such as fremanezumab (23) and galcanezumab (24), or against CLR, such as erenumab (25), have been approved by the Food and Drug Administration (FDA) for the treatment of migraine. Non-peptide antagonists of CLR, such as ubrogepant (26,27), were also approved by the FDA, and rimegepant has been through phase III clinical trials (28,29) for the treatment of migraine. These antibodies and antagonists have been useful for the study of the CGRP/CLR axis.

3. CGRP and cancer-associated pain

Pain is one of the most common factors affecting the quality of life in patients with cancer and 75-95% of patients with metastatic or advanced-stage cancer will experience considerable amounts of cancer-induced pain (30). The incidence rate of cancer related pain varies for different types of cancers. For instance, breast adenocarcinoma is frequently characterized as being painful only after it metastasizes to the bone (31). However, melanomas (even in the head and neck) are typically not painful at the primary site or metastatic sites (32). In OSCC, pain is the most frequent complaint at the primary site (9), and

the prevalence and intensity of OSCC-associated pain are higher than in all other types of cancer (10,11). In a prospective study, the pretreatment pain in OSCC has been able to predict peripheral nerve invasion (33), and was identified as an independent predictor of survival in 2,340 patients with OSCC in a retrospective study (10). The dorsal root ganglion (DRG) and TG neurons are pseudounipolar, which means that the axon bifurcates and sends projections to both the dorsal horn of the spinal cord or brainstem, and to the periphery (34). This unique morphology allows the release of transmitters at central and peripheral sites, and allows for bidirectional communication between the two terminals (35). When peripheral noxious stimuli (including high hydrogen, high temperature, capsaicin or prostaglandin E₂) act on peripheral nociceptors, CGRP serves a dual role in afferent and efferent nerves (36). On one hand, CGRP is released in the synapse of primary afferent nerves through anterograde axoplasmic transport and acts on its receptors on the postsynaptic neurons in the DRG and TG, thus transmitting algnesia to pain centers. On the other hand, it is also released from peripheral nerve terminals and exerts paracrine effects on the surrounding tissues by retrograde axoplasmic transport (36).

CGRP modulates pain in two major ways. A relatively well-researched mechanism is that following the vasodilation effect of CGRP, the accumulated proinflammatory factors irritate peripheral nociceptors. In addition, CGRP may directly influence peripheral and central sensitization, resulting in hyperalgesia and allodynia (37) (Fig. 2). A recent study demonstrated that CGRP induces differential regulation of cytokines, mostly interleukin (IL)-1 β and IL-6, from satellite glial cells in TG, causing increased pain (37). In most situations, these two effects often co-exist and mutually promote each other (36).

The present review summarized the existent studies on the role of CGRP in cancer-associated pain (Table SI). Most studies used *in vivo* experiments to reveal that there are higher numbers of CGRP(+) sensory nerves sprouting adjacent to an ectopic cancer and in the DRG or TG than elsewhere, and mechanical allodynia and thermal hyperalgesia exist in various types of tumor, such as gingival cancer, metastatic bone cancer and breast cancer (35,38-45). Animal models should be optimized to study the role of CGRP in OSCC-associated pain. Nagamine *et al* (44) established a rat model of oral cancer pain by inoculating cancer cells into the lower gingiva, and instead of hind paw withdrawal, used head withdrawal as the evaluation index; in addition, it was revealed that the mechanical allodynia and thermal hyperalgesia in the ipsilateral maxillary and mandibular nerve area were accompanied by nerves with upregulated CGRP expression in the TG. Although this was an innovative discovery, perhaps adding another assessment index, such as food intake, may help to confirm this result.

Overall, CGRP exerts considerable roles in cancer-associated pain, as well as in OSCC. The active sites of CGRP are the peripheral nociceptors and central nerve endings in the DRG or TG. However, the current *in vivo* models and antagonists require further improvements.

4. CGRP in cancer development

Initially, high levels of CGRP were identified in tumor tissues and serum of patients with medullary thyroid carcinoma (46).

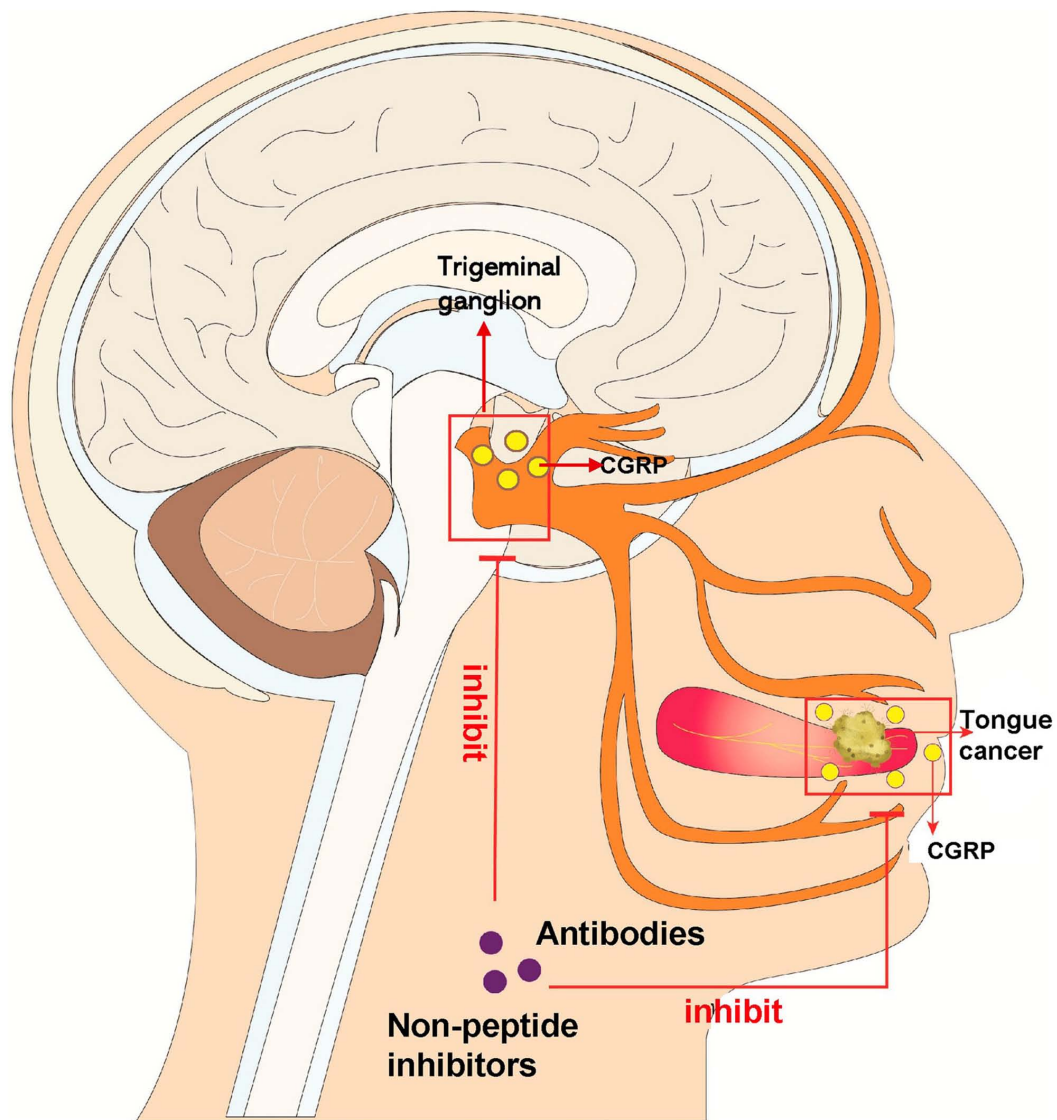


Figure 1. CGRP: A bridge target between cancer development and cancer-associated pain in OSCC. CGRP release may mediate pain and cancer development simultaneously. On one hand, CGRP released from peripheral nerve terminals can exert paracrine effects on the surrounding tissues including cancer cells. On the other hand, CGRP can act on its receptors on the postsynaptic neurons in the TG, thus transmitting algnesia to pain centers. Future studies should investigate the strategy of using antibodies or antagonists targeting the CGRP-calcitonin receptor-like receptor axis approved by the Food and Drug Administration for OSCC treatment, as this may affect both cancer development and cancer-associated pain. CGRP, calcitonin gene-related peptide; OSCC, oral squamous cell carcinoma.

Subsequent studies emphasized the diagnostic value of CGRP in non-small cell lung carcinoma (47,48), neuroendocrine tumors (49), prostate cancer (50,51) and particularly medullary thyroid carcinoma (46,52). Recently, Angenendt *et al* (53) analyzed the expression levels of the gene encoding the CLR, *CALCRL*, in >1,500 patients with well-characterized acute myeloid leukemia from five international cohorts, revealing that increasing transcript levels of *CALCRL* were associated with decreasing complete remission rates, 5-year overall survival and event-free survival. Furthermore, CRISPR-Cas9-mediated knockout of *CALCRL* markedly impaired colony formation in human myeloid leukemia cell lines (53).

One way in which CGRP is able to influence tumor behavior is secondary to its potent vasodilation effect. A previous study on CGRP-knockout mice revealed that endogenous CGRP facilitated tumor-associated angiogenesis and tumor growth in Lewis lung carcinoma-bearing mice and that the admin-

istration of the CGRP antagonist CGRP8-37 or denervation markedly suppressed tumor growth and tumor-associated angiogenesis (54).

A number of studies have revealed that CGRP can directly modulate development of various types of cancer, with a large amount of literature reporting the expression of the CGRP receptor complex in cancer cells (55-57). CGRP was demonstrated to be able to modify the chemokinetic abilities of a metastatic breast cancer cell line and increase the expression of its receptors (58). In addition, it has been demonstrated that CGRP increased the invasive ability of prostate cancer PC-3 cells and an osteosarcoma cell line (13,50). Recently, Dallmayer *et al* (56) revealed that targeting the CALCB/RAMP1 (the receptor complex of β -CGRP) axis inhibited the growth of Ewing sarcoma cells. Despite the lack of studies on the role of CGRP in OSCC, several studies have revealed a wide distribution of CLR/RAMP1 in oral soft

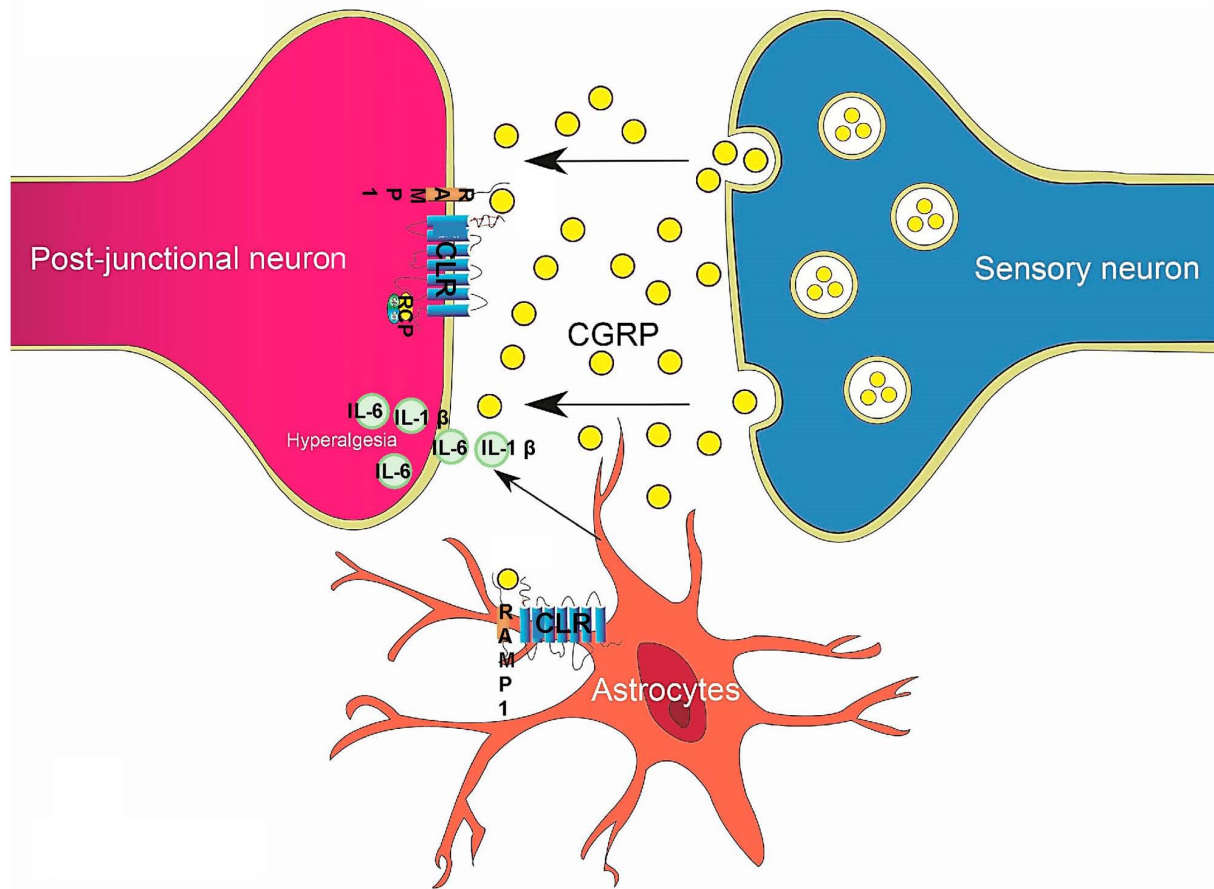


Figure 2. CGRP in pain conduction and hyperalgesia. CGRP exerts its pain conduction role via binding to the CLR/RAMP1/RCP receptor complex on post-junctional neurons. In addition, CGRP binds to receptors on astrocytes, releasing IL-6 and IL-1 β , resulting in hyperalgesia. CGRP, calcitonin gene-related peptide; CLR, calcitonin receptor-like receptor; RAMP1, receptor activity-modifying protein 1; RCP, receptor component protein; IL, interleukin.

tissues, bones and dental tissues (59). Therefore, the present review discussed the mechanisms that CGRP may utilize in cancer development.

Aerobic glycolysis. It is well known that rapidly growing cancer cells tend to utilize glycolysis during proliferation, regardless of oxygen availability, which is known as the Warburg effect (60). Aerobic glycolysis is one of the hallmarks of cancer, including OSCC (61). Various enzymes participating in glycolysis are upregulated in OSCC and are associated with poor prognosis (62-64). Rossetti *et al* (65) demonstrated that CGRP potentially antagonizes the effect of insulin in glycogen synthesis and enhances glycolysis in muscles, which was attributed to the activation of cyclic adenosine monophosphate (cAMP). However, a recent study on insulin resistance revealed that with the activation of transient receptor potential vanilloid-1 proteins/CGRP axis, glucose transporter 4 expression was upregulated at the protein and mRNA levels, which ameliorated insulin resistance (66). Despite the disparity of certain results, these findings suggest that CGRP may serve important roles in cell glycometabolism.

A pivotal signaling pathway in cell glycometabolism in which CGRP participates is the AMP-activated protein kinase (AMPK) signaling pathway (67). AMPK senses the intracellular AMP/adenosine triphosphate (ATP) or

adenosine diphosphate/ATP levels, promoting metabolic pathways that generate ATP, such as glycolysis and lipolysis, and inhibiting those consuming ATP, such as glycogen and lipid synthesis (68). Danaher *et al* (67) suggested that CGRP activates AMPK through phosphorylation of Thr172 in its α -subunit. As a serine/threonine protein kinase that works as a sensor of cellular energy, phosphorylated AMPK is able to directly phosphorylate proteins or modulate gene transcription involved in glucose and lipid metabolism, which increases the cell utilization of glucose (68).

Lipid metabolism. Lipid metabolism is also reprogrammed in different types of cancer, but not in the same way for all. Multiple lines of evidence suggest that CGRP may regulate lipid metabolism in physiological and pathological conditions. Compared with normal-weight females, the plasma CGRP concentration is increased in obese females (body mass index >35 kg/m²) (69). In pre-obese Zucker rats, the fasting plasma CGRP levels were elevated in lean animals prior to the appearance of body weight differences compared to control group (70). Furthermore, CGRP-knockout mice were observed to be protected from diet-induced obesity compared with CGRP^{+/+} mice, without any obvious attenuation of food intake (70). Danaher *et al* (67) suggested that levels of CGRP similar to those in the blood markedly stimulated fatty acid

β -oxidation and evoked concomitant mobilization of muscle lipid via receptor-mediated activation of muscle lipolysis in rodents. In OSCC, it was demonstrated that fenofibrate, a particularly potent clinical lipid-lowering agent, suppressed oral tumorigenesis and cancer development by downregulating mTOR activity and activating the AMPK signaling pathway (71). In another retrospective study including 576 patients diagnosed with T1/2N0M0 OSCC without any weight loss prior to diagnosis, the progression-free survival time was poorer in obese patients than in those of normal weight (72). Future studies should investigate whether CGRP may regulate lipid metabolism in OSCC.

Aberrant internalization and intracellular trafficking. It is normal for the internalization of receptors to serve as a physiological feedback mechanism to switch off the initiated cellular signaling pathways. However, studies on certain GPCR-like receptors, such as the thyroid-stimulating hormone (TSH) receptor and the parathyroid hormone receptor, have demonstrated that an internalized ligand-receptor compound is able to convey sustained signals in endosomes (73,74). The CLR/RAMP1 receptor complex is internalized together into an endosome after combining with CGRP (75). Yarwood *et al* (76) revealed that endosomal CLR is able to activate protein kinase C in the cytosol, as well as ERK in the cytosol and nucleus. In addition, the authors developed a cholestanol-conjugated antagonist (CGRP8-37-cholestanol) to prevent sustained neuronal excitation caused by CGRP, which accumulates in endosomes and is capable of inhibiting endosomal CLR/cAMP signaling without affecting CLR/RAMP1 receptors at the cell surface. A similar result was obtained in a study on substance P, which is frequently regarded as coexisting with CGRP (77,78). A number of studies agree that endosomal signaling is more efficient compared with the traditional plasma membrane signaling and may result in tumorigenesis (76,79). Godbole *et al* (79) demonstrated that the TSH receptor co-internalizes with TSH and traffics retrogradely to the trans-Golgi network, where it activates an endogenous pool of G_s proteins; this leads to a delayed phase of local cAMP production and protein kinase A activation at a critical position near the nucleus, which appears to be required for efficient phosphorylation and gene transcription of the cAMP response element-binding protein.

Overall, the aberrant internalization of receptors contributes to tumorigenesis. CLR/RAMP1 may be sustained and activated through an endosomal signaling pathway, which is an unexploited but promising field in OSCC. Cholestanol-conjugated antagonists may represent a novel approach for receptor antagonist development.

Immune evasion. Immune evasion is another hallmark of cancer (80). Previous studies have demonstrated that the aforementioned effects may be at least partially mediated by CGRP. Anatomically, the peripheral nervous system is connected with lymphoid organs through sensory nerves (81,82). In addition to sensory nerves, CGRP may be produced by immune cells, including T cells (83,84), B cells (85) and monocytes (86). The CLR/RAMP1 receptor appears to be expressed by most immune cells (87,88). Endogenous CGRP from nerves or immune cells is a major suppressor of immune reac-

tions (89,90). In differentiated CD4⁺ T cells, CGRP inhibits the production of tumor necrosis factor- α and interferon- γ by T helper type 1 cells via elevating intracellular cAMP levels, while IL-4 production by T helper type 2 cells is not affected (89,90). Following treatment of Langerhans cells with CGRP, the capacity of these cells to stimulate the proliferation of murine T cells was impaired (91). Similarly, treating human monocytes or dendritic cells with CGRP markedly compromised the proliferative response of allogeneic T cells through the release of IL-10 (92). The immunomodulatory roles of CGRP are explained more in detail in a previous review (93).

In OSCC, an impaired and suppressed immune function in both the whole human system and in the local tumor microenvironment is associated with poor prognosis for patients with OSCC (61). Future studies should analyze how CGRP may regulate immune reactions in OSCC.

5. Conclusion and future directions

Clinical and pathological features confirm that nerves serve an important role in OSCC, as well as in other types of cancer. However, sensory nerves, rather than autonomic nerves, appear to have a pivotal role in OSCC. CGRP is mostly expressed in sensory neurons in the TG and DRG (94,95). CGRP exerts a significant role in both cancer development and cancer-associated pain. CGRP promotes cancer development through metabolic reprogramming, anomalous receptor internalization or inhibition of antitumor immune responses. In addition, CGRP may augment pain by inducing mechanical allodynia peripherally and centrally. Based on the current knowledge, the present review suggested that CGRP may represent an important bridge between cancer development and cancer-associated pain in OSCC, and this hypothesis should be further investigated in future studies.

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Availability of data and materials

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Authors' contributions

YZ, TJ and XW conceived and designed the study. CL researched the literature. YZ wrote the manuscript. CL and XW designed the figures. YZ, CL, XW and TJ revised and edited the manuscript. All authors read and approved the final manuscript.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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