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Mitogen-activated protein kinase signaling pathway in oral cancer (Review)

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Abstract. The mitogen-activated protein kinase (MAPK) signaling pathway is associated with tumor cell proliferation, differentiation, apoptosis, angiogenesis, invasion and metastasis. The present review assesses the involvement of the MAPK signaling pathway in oral cancer progression and invasion based on analysis of individual sub-pathways and their mechanisms of action. The regulation of this pathway for targeted oral cancer therapy is explored and the challenges confronting this, as well as corresponding potential solutions, are discussed. Exploring this pathway with an emphasis on its components, subfamilies, sub-pathways, interactions with other pathways and clinical practice modes may improve oral cancer treatment.

Contents

1. Introduction

Head and neck malignant carcinoma is a commonly diagnosed cancer and its incidence has risen over the past decade (1). Oral squamous cell carcinoma (OSCC) is an important oral malignancy, accounting for >90% of head and neck malignant carcinoma cases (2). It has a 5-year survival rate of ~50% (3), due to delayed diagnosis, disease recurrence, distant metastasis and therapeutic resistance. At present, multiple attempts are being made to develop efficient targeted therapies, which often involve the use of various inhibitors for improving treatment due to their special advantages, such as low cytotoxicity, strong specificity and long duration of action.

Targeted therapies aim at controlling the signaling pathways through which cell survival and death are regulated. Multiple signaling pathways may function in oncogenesis and in the growth of oral malignancies (4,5). Among these is the mitogen activated protein kinase (MAPK) signaling pathway, which regulates the expression of a large number of proteins involved in the control of cell proliferation, differentiation and apoptosis (6,7), and remains a focus of investigation for therapeutic targeting, along with the development of appropriate inhibitors (8). These inhibitors have been demonstrated to be effective for the treatment of oral cancer and other malignancies, including human colon cancer, breast cancer and lung cancer (9-12). Certain inhibitors, which have been examined in various clinical trials, are being used to treat patients with oral cancer and are eliciting promising results. The purpose of the present review is to provide an update on the involvement of the MAPK pathway in oral cancer progression and invasion, and to review the progress made in the development of pharmacologic inhibitors to regulate the MAPK signaling pathway, in order to improve oral cancer treatment.

2. MAPK signaling sub-pathways

The MAPK signaling pathway is comprised of four sub-pathways, namely the extracellular signal-regulated kinase (ERK)1/2) sub-pathway, the c-Jun N-terminal kinase (JNK) sub-pathway, the p38 sub-pathway and the ERK5 sub-pathway (13). These sub-pathways correspond to the ERK1/2, JNK1/2/3, p38α/β/γ/δ and ERK5 subfamilies of MAPK, respectively, and are a chain of proteins that transduce various extracellular signals to the nucleus, controlling gene expression through transcriptional factors (14). By regulating protein activities through signaling cascades that consist of MAPK kinase kinase (MAPKKK), MAPK kinase and MAPK (15), these sub-pathways impact tumor cell proliferation, differentiation and survival (16,17), as presented in Fig. 1.

The ERK1/2 sub-pathway has received the most attention thus far (18-20). It has 3 important components: Ras, Raf and

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MEK. As shown in Fig. 1, in response to the binding of growth factors and hormones to cell surface receptors (21), the level of Ras-guanosine triphosphate (GTP) increases in cells, which in turn promotes kinase activation. The GTP-bound forms of Ras directly bind and thus recruit cytosolic dimers of the Raf kinases to the plasma membrane (22). Once localized at the membrane, Raf is activated through phosphorylation by other kinases or autophosphorylation. It then phosphorylates and activates MEK, which subsequently induces the phosphorylation and activation of ERK. The activated ERK1/2 dissociates from the Ras-Raf-MEK-ERK1/2 complex and phosphorylates a number of cytoskeletal proteins, kinases, and transcription factors, including nuclear factor (NF)-κB, AP-1, ETS-1, c-Myc, and members of the signal transducer and activator of transcription family (23,24). The functional consequences of substrate-level phosphorylation by ERK1/2 include changes in cellular motility and gene expression that promote proliferation, differentiation, cellular survival, immortalization and angiogenesis (25).

The JNK sub-pathway is associated with activation of MAPKKK [mitogen activated protein kinase kinase kinase (MEKK)1/2/3/4], a response induced by a number of environmental stresses (including osmotic stress, UV light, heat shock and growth factor withdrawal) and cytokines [including tumor necrosis factor (TNF)-α and interleukin (IL)-β] (26). The activated MEKK1/2/3/4 in the sub-pathway phosphorylates a series of proteins in the following order: Mitogen activated protein kinase kinase (MKK) 4/7→JNK1/2/3→c-Jun, activating transcription factor (ATF)-2, and ELK1, ETS transcription factor. The sub-pathway contributes to cell growth, differentiation, apoptosis and survival through stress/cytokine-sensitive responses (27).

Similar to the JNK sub-pathway, the p38 sub-pathway is associated with the response of MAPKKK [mixed lineage kinases (MLKs), mitogen activated protein kinase kinase kinase 7 (TAK-1) and mitogen activated protein kinase kinase kinase 5 (ASK-1)] caused by stress and pro-inflammatory cytokines, including IL-1 and TNF-α (28). The activated MLKs, TAK-1 and ASK-1 in this sub-pathway phosphorylate and activate various kinases sequentially, from MLKs, TAK-1, ASK-1 to MKK3/6 to the four p38 isoforms (p38α/β/γ/δ). The activated p38 isoforms then activate the substrates ATF-2, cAMP response element-binding protein, DNA damage inducible transcript 3 and myocyte enhancer factor (MEF)2C. Cell differentiation, apoptosis, survival and inflammation are induced by this pathway (29).

In comparison with the other sub-pathways, the ERK5 sub-pathway has been less intensively investigated (30). It is associated with a variety of stimuli, including mitogens (for example, epithermal growth factor) and cellular stresses (including oxidation and osmotic stress) (31). Its activation is consistent with the cascade MEKK2/3→MKK5→ERK5→MEF2A/C/D AP-1 transcription factor subunit (C-Fos) and Fos related antigen-1, and the ERK5 sub-pathway regulates cell growth, differentiation and survival (32,33).

3. Action mechanisms of the MAPK signaling pathway in oral cancer

The MAPK signaling pathway is involved in various cellular responses, including cell proliferation, apoptosis, angiogenesis, cell migration and metastasis. The deregulation of MAPK signaling leads to inappropriate responses, induced through abnormal gene expression. Further elucidation of the mechanisms underlying the action of proteins that regulate MAPK signaling and of their expression patterns in oral cancer may provide novel insights into the signal transduction mechanisms in cell cycle progression and malignant transformation. A large number of proteins serve as targets that act on the oncogenesis and growth of oral tumor cells. These targets include angiopoietin-like 3, annexin A10, SH3 domain containing kinase binding protein 1, quaking 5, epidermal growth factor receptor, fibroblast activation protein, parathyroid hormone-related protein and 70-kDa ribosomal S6 kinase (34-41). Their functions and molecular mechanisms in the MAPK signaling pathway that concern oral cancer are summarized in Table I.

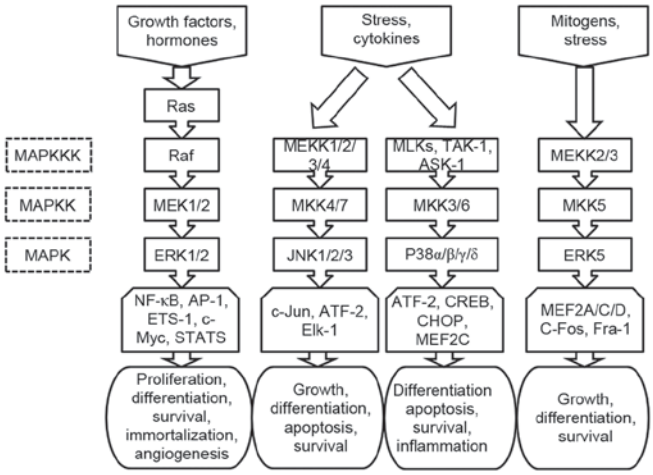

Figure 1. Schematic illustration of MAPK signaling pathway, where each MAPK phosphorylates several transcriptional factors, regulating protein expression.

Promoting tumor cell proliferation. Within the MAPK signaling pathway, two main mechanisms are responsible for promoting tumor cell proliferation. The primary mechanism is associated with early gene-encoded c-Jun and C-Fos proteins, which constitute transcription factor AP-1 in the form of a heterodimer or homodimer following MAPK phosphorylation; for example, ERK phosphorylation (18). These factors then combine with corresponding promoter regions of DNA and upregulate the expression of cyclin D1, which promotes the progression of the cell cycle from the G1 phase to the S phase and accelerates cell proliferation. The other mechanism is attributed to the function of activated MAPK, which suppresses expression of cell cycle inhibitory proteins including $p27^{KIP}$, attenuating the inhibition of the cell cycle (42).

Inhibiting tumor cell apoptosis. There are three main mechanisms of the MAPK signaling pathway that inhibit tumor cell apoptosis. Firstly, the signaling pathway may directly suppress the activity of the apoptosis end effector, caspase-3, which inhibits hydrolysis of tubulin to maintain the integrity of its spindle body. This function prevents cell apoptosis from being induced by various stimuli. Secondly, the pathway may

indirectly suppress the activity of caspase-3 by activating the inhibitors of apoptosis molecules, including inhibitor of apoptosis and B cell lymphoma-2 family proteins (43,44). Thirdly, the pathway may block the release of mitochondrial cytochrome C and interfere with the activation process of upstream caspase-9, indirectly decreasing the activity of downstream caspase-3 (45).

Promoting tumor angiogenesis. Tumor angiogenesis not only provides nutrition for increasingly active tumor cell division, but is also involved in the outward expansion of the tumor. A number of vascular growth factors upregulate the expression level of vascular endothelial growth factor via activation of the MAPK signaling pathway, resulting in angiogenesis (38).

Inducing tumor invasion and metastasis. The process of tumor invasion relies on the precise coordination of extracellular matrix adhesion and hydrolysis in association with two members of the matrix metalloproteinase (MMP) family; namely MMP-2 and MMP-9. The expression of MMPs is primarily controlled by MAPK and other signaling pathways, including the Janus kinase (JAK)/signal transducer and activator of transcription (STAT) pathway (46). This has been validated by a number of studies in which the inhibitory functions of anticancer drugs on tumor invasion and metastasis were demonstrated to occur by downregulating the MAPK signaling pathway, which resulted in reduced expressions of MMPs (47,48).

4. Regulation of the MAPK signaling pathway for targeted oral cancer therapy

The expression levels of the component proteins of the MAPK signaling pathway tend to increase in oral cancer (49). Activation of the MAPK signaling pathway is known to cause the transformation of normal cells to tumor phenotype (6). Thus, inhibition of this pathway may enable restoration of the tumor cells to the untransformed phenotype *in vitro*, thereby inhibiting the growth of the tumor in the body (50,51). Theoretically, intervention targeting any component in the MAPK signaling pathway has the potential to arrest tumor growth. For this reason, multiple inhibitors have been developed to target different components of the pathway (52-55). These inhibitors serve as promising, effective antineoplastic agents for the treatment of malignant oral tumors, and are characterized by low cytotoxicity, strong specificity, a long duration of action, high solubility and metabolic stability, all of which contribute to their anti-tumor effects. At present, certain inhibitors have been used in phase I or phase II clinical trials and achieved promising effects (55).

These inhibitors may inhibit oral tumor cell proliferation by blocking the activation of corresponding target molecules of the MAPK signaling pathway. They include epigallocatechin-3-gallate, aliphatic acetogenin, galanin receptor 1, and S-allylcysteine (56-59). Certain inhibitors, including pterostilbene, astaxanthin and sulfasalazine (SSZ) (60-62), may also induce tumor cell apoptosis. Other inhibitors include those that have the potential to inhibit either oral tumor angiogenesis (for example, cetuximab) (63) or tumor invasion and metastasis (for example, vinculin, phenethyl isothiocyanate, cardiotoxin III and resveratrol) (64-67). Their characteristics are listed in Table II.

Besides the aforementioned inhibitors, it is worth noting that certain inhibitors may induce autophagic cell death via activation of the MAPK signaling pathway. For example, high-concentration SSZ was revealed to initially trigger autophagy, which then induced apoptosis in HSC-4 cells by activating the ERK1/2 sub-pathway. Therefore, SSZ may have the potential to treat oral cancer (62).

5. Discussion

Although a number of genetic and experimental observations have validated that inhibitors of MAPK cascades would act as effective antineoplastic agents for the treatment of oral cancer with approved performance, there are a few of remaining challenges and questions to be resolved.

First, mutated members of the cascade, including Ras and Raf, are expected to serve as inhibitory targets in the MAPK pathway (68,69), but their functions have not been sufficiently illustrated. The most commonly observed mutations are those that arise in the Ras genes, including H-Ras, N-Ras and K-Ras, with corresponding mutations being detected in >30% of patients with OSCC in Southeast Asia, primarily caused by chewing tobacco (70). These Ras genes have the potential to regulate the MAPK and phosphoinositide 3-kinase (PI3K)/protein kinase B (Akt) pathways, and a Ras mutation may therefore activate both of them. As for Raf, its activity in certain cell types is negatively regulated by Akt, revealing the presence of cross-talk between the two pathways (71). Activating mutations in the B-Raf gene (one of the three Raf genes, which include A-Raf, B-Raf and C-Raf) were also identified in oral cancer (19,72). Unlike mutation of Ras, mutation of B-Raf only causes activation of the MAPK signaling pathway. It functions independently in promoting the engagement of the MAPK cascade (73). These findings confirm the complicated functions of the targets in the MAPK pathway, indicating that different inhibitors of the same target and drugs for different targets within the same pathway may demonstrate marked differences in effectiveness, depending on tumor type and mutational status. Hence, improved understanding of their biochemical properties or modes of action, along with improvements in their pharmacologic profiles, will enable more optimal treatment of oral cancers through the development of more effective therapies.

Secondly, inhibiting only a single subfamily or sub-pathway has been revealed to be insufficient to promote apoptosis or arrest growth to control oral cancer, due to a potential compensation effect of alternative subfamilies or sub-pathways (13). The occurrence of most oral cancer is associated with dysregulation of multiple subfamilies and sub-pathways. Furthermore, certain sub-pathways, including the ERK5 sub-pathway, have not been sufficiently explored and their cross-functions remain unclear. From this perspective, clearly identifying the functions and interactions of these signaling transduction sub-pathways is critical to inducing tumor cell apoptosis and overcoming tumor resistance. This is the clinical need that must be satisfied in order to improve treatment for oral cancer, and therefore warrants additional attention.

Table I. Therapeutic targets of the MAPK signaling pathway for oral cancer treatment.

Author, year	Classification	Target name	Target description	Target functions	Molecular mechanisms in MAPK signaling pathway	Effects	(Refs.)
Koyama *et al*, 2015	Promoting cell proliferation	ANGPTL3	A secretory glycoprotein	Regulating cell proliferation and promoting blood vessel formation	The proliferation significantly decreases in ANGPTL3 knockdown cells due to inactivated ERK and cell-cycle arrest at the G1 phase resulting from upregulation of CDK inhibitors, including $p21^{Cip1}$ and $p27^{Kip1}$. There is a marked reduction in the growth of ANGPTL3 knockdown-cell xenografts, with decreased levels of phosphorylated ERK relative to control-cell xenografts	A potentially useful diagnostic and therapeutic target for controlling proliferation of oral cancer	(34)
Shimizu *et al*, 2012		ANXA10	A calcium and phospholipid binding protein	Impacting endocytosis and exocytosis; having anticoagulant activity; interacting with cytoskeleton; differentiation; cellular proliferation	The ANXA10-knockdown cell proliferation is decreased due to inactivation of ERK and cell-cycle arrest at the G1 phase, associated with upregulation of CDK inhibitors	Serving as an indicator of cellular proliferation and a potential therapeutic target for developing new OSCC treatment regimens	(35)
Wakasaki *et al*, 2010		CIN85	A widely expressed, multifunctional adaptor protein consisting of three N-terminal SH3 domains	Promoting the EGFR-mediated cancer cell growth pathway	CIN85 promotes TGF-α-induced activation of Ras and phosphorylation of downstream molecules including c-Raf, MEK and ERK, upregulating expression of c-Myc that is critical for sustained proliferation of OSCC	Serving as an attractive therapeutic target for controlling proliferation of OSCC	(36)
Fu and Feng, 2015		QKI-5	An RNA-binding protein	Suppressing tumor proliferation	The underexpression of tumor suppressor QKI-5 activates the MAPK signaling pathway and contributes to uncontrolled cyclin D1 expression, resulting in increased proliferation of oral cancer cells	Serving as a diagnostic and therapeutic target for controlling proliferation of oral cancer cells	(37)
Williams, 2010	Promoting tumor angiogenesis	EGFR	A member of the tyrosine kinase family of receptors	Enhancing cell motility, altering cell adhesion and promoting angiogenesis	The downstream signaling activated by EGFR includes STAT1/3 and PI3K which activates Akt, Ras-Raf-MEK-ERK1/2 signaling and PLC-γ, contributing to cell survival, proliferation, and angiogenesis in OSCC	Serving as a potential therapeutic target for controlling cell proliferation and angiogenesis in OSCC	(38)

Table I. Continued.

Author, year	Classification	Target name	Target description	Target functions	Molecular mechanisms in MAPK signaling pathway	Effects	(Refs.)
Wang *et al*, 2014	Promoting cell migration and metastasis	FAP	A homodimeric integral membrane gelatinase belonging to the serine protease family	Regulating cell proliferation, adhesion, migration, invasion, and metastasis	The knocking down endogenous FAP suppresses cell proliferation, adhesion, migration, invasion, and metastasis by inactivating PTEN/PI3K/Akt and Ras-Raf-MEK-ERK1/2 signaling. This in turn inhibits GSK-3β and its downstream signals including cell-cycle regulators, EMT and MMPs in OSCC	Serving as a potential therapeutic target for controlling proliferation, invasion and metastasis of OSCC	(39)
Yamada *et al*, 2008		PTHrP	A stimulator of osteoclastic bone resorption	Increasing cell proliferation, survival, adhesion, migration, and invasion	PTHrP is upregulated by EGF stimulation via ERK1/2 and p38 sub-pathways. The PTHrP silencing by EGFR inhibitor AG1478 treatment suppresses cell proliferation, migration, and invasiveness; the combined treatment with AG1478 and PTHrP knockdown achieves synergistic inhibition of malignant phenotypes	Serving as a target for the suppression of proliferation, migration, and invasion of oral malignancies	(40)
Wu *et al*, 2016		p70S6K	A serine/threonine kinase, belonging to the AGC-kinase family	Promoting cell growth and metastasis	The IL-6-induced p70S6K activation is attenuated by inhibitors of the PI3K/Akt/mTOR, MAPK, and JAK/STAT3 signaling pathways, suggesting that it is located downstream of these pathways. p70S6K promotes IL-6-induced epithelial-mesenchymal transition and metastasis of OSCC	Serving as a target for inhibition of oral tumor growth and metastasis	(41)

MAPK, mitogen-activated protein kinase; ANGPTL3, angiopoietin-like 3; ERK, extracellular signal-regulated kinase; CDK, cyclin-dependent kinase; ANXA10, annexin A10; OSCC, oral squamous cell carcinoma; CIN85, Sc-Cbl-interacting protein of 85 kDa; EGFR, epidermal growth factor receptor; TGF-α, transforming growth factor- α; MEK, MAPK/ERK kinase; QKI-5, quaking 5; STAT, signal transducer and activator of transcription; PI3K, phosphoinositide 3-kinase; Akt, protein kinase B; PLC-γ, phosphoinositide phospholipase C; FAP, fibroblast activation protein; PTEN, phosphatase and tensin homolog; GSK-3β, glycogen synthase kinase-3β; EMT, epithelial-mesenchymal transition; MMP, matrix metalloproteinase; PTHrP, parathyroid hormone-related protein; EGF, epidermal growth factor; IL, interleukin; p70S6K, 70-kDa ribosomal S6 kinase; mTOR, mammalian target of rapamycin; JAK, Janus kinase.

Table II. Target inhibitors of the MAPK signaling pathway for oral cancer treatment.

Author, year	Classification	Target inhibitor	Inhibitor description	Inhibitor functions	Molecular mechanisms in MAPK/ERK pathway	Effects	(Refs.)
Lee *et al*, 2015	Inhibiting cell proliferation	EGCG	A biologically active polyphenol in green tea	Inhibiting cell growth and inducing cell apoptosis	EGCG attenuates cell proliferation and arrests cell cycle at the G1 phase by upregulating BTG2 expression via p38 and ERK1/2 sub-pathways in OSCC cells	Serving as a new preventive agent for patients with OSCC	(56)
D'Ambrosio *et al*, 2011		Aliphatic acetogenin	A class of compounds, mainly including: Compound 1 [(2S,4S)-2,4-dihydroxyheptadec-16-enyl acetate] and Compound 2 [(2S,4S)-2,4-dihydroxyheptadec-16-ynyl acetate]	Inhibiting cancer cell proliferation and growth	The Compounds 1 and 2 synergistically inhibit phosphorylation of c-RAF (Ser338) and ERK1/2 (Thr202/Tyr204). Only Compound 2 prevents EGF-induced activation of EGFR (Tyr1173) via the ERK1/2 sub-pathway	The molecular mechanism of aliphatic acetogenin indicates that targeting multiple molecules in the ERK1/2 sub-pathway simultaneously may offer more effective prevention and treatment of oral cancer compared with using specific inhibitors that target only one of the molecules in the pathway	(57)
Henson *et al*, 2005		GALR1	A G-protein-coupled receptor	Regulating cell proliferation	GALR1 inhibits proliferation in immortalized and malignant keratinocytes by inactivating the MAPK signaling pathway. The inhibitory effects on proliferation in epithelial cells raise the the possibility that its inactivation or dysregulation may lead to uncontrolled proliferation and neoplastic transformation	Serving as a prognostic biomarker and a treatment option	(58)
Tang *et al*, 2009		SAC	A water soluble garlic extract	Having anticarcinogenic effects that inhibit tumor cell growth	SAC effectively inhibits the proliferation, upregulates the expression of E-cadherin and stabilizes the E-cadherin/β-catenin adherent junction complex in OSCC cells, partially through the suppression of MAPK signaling pathway and downregulation of the SLUG repressor protein	Serving as a potential anticancer agent with improved selectivity toward OSCC cells	(59)
Ko *et al*, 2015	Inducing apoptosis	Pterostilbene	A naturally occurring phytoalexin	Having antioxidant activity, cancer prevention activity, and cytotoxicity	Pterostilbene suppresses cell growth and induces apoptosis in SAS and OECM-1 cell lines by inhibiting Akt, p38 and ERK1/2 and activating the JNK sub-pathway	Serving as a novel and promising agent for treating oral cancer	(60)

Table II. Continued.

Author, year	Classification	Target inhibitor	Inhibitor description	Inhibitor functions	Molecular mechanisms in MAPK/ERK pathway	Effects	(Refs.)
Kavitha *et al*, 2013		Astaxanthin	An antioxidant carotenoid	Having anti-inflammatory and anticancer properties; modulating immune response and oxidative stress	Astaxanthin inhibits NF-κB and Wnt/β-catenin signaling via inactivation of the MAPK and PI3K/ Akt signaling pathways to induce intrinsic apoptosis	Serving as a promising agent for prevention and therapy of oral cancer	(61)
Han *et al*, 2014		SSZ	A drug composed of sulfapyridine and mesalazine	Having anti-inflammatory properties; inducing apoptosis	SSZ induces autophagic cell death in HSC-4 cells by inhibiting Akt and activating the MAPK signaling pathway	Serving as an effective chemotherapeutic agent for the treatment of OSCC	(62)
Psyrri et al, 2014	Inhibiting angiogenesis	Cetuximab	A chimeric IgG1-human monoclonal antibody against the extracellular domain of EGFR apoptosis	Inhibiting cell cycle progression, angiogenesis and metastasis; inducing apoptosis	Cetuximab inhibits angiogenesis of OSCC by inactivating the ERK1/2 sub-pathway and/or PI3K/ Akt signaling pathway	Serving as a potential therapeutic agent for the treatment of OSCC	(63)
Yoshimoto et al, 2014	Inhibiting cell migration and metastasis	Vinculin	A cellular adhesion protein	Inhibiting cell migration	The upregulation of MT1-MMP transcription by vinculin knockdown is abrogated by ERK inhibition. MT1-MMP may facilitate further HSC-4 cell survival, induced by vinculin knockdown	Serving as a key agent for controlling OSCC cell migration through the MAPK signaling pathway	(64)
Chen et al, 2013		PEITC	A member of the isothiocyanate family	Arresting cell cycle and stimulating apoptosis; inhibiting migration and invasion	PEITC is able to inhibit the invasion of EGF-stimulated SAS oral cancer cells by targeting EGFR and its downstream MAPK signaling molecules, reducing expression and enzymatic activities of MMP-2 and MMP-9	Serving as a promising therapeutic agent for the treatment of oral cancer metastasis	(65)
Yen et al, 2013		CTXIII	A protein composed of 60 basic amino acid residues	Inhibiting cellular proliferation and inducing apoptosis	The CTXIII treatment leads to downregulated protein expression of MMP-2 and MMP-9, and the phosphorylation of JNK and p38 is increased independent of ERK phosphorylation	Serving as a potential agent for oral cancer therapy	(66)
Lin et al, 2015		Resveratrol	A polyphenolic compound	Having antioxidative, cardioprotective, and neuroprotective properties	Resveratrol inhibits TPA-induced MMP-9 gelatinolytic activity and protein expression, as well as phosphorylation of JNK1/2 and ERK1/2, involved in downregulating protein expression and the transcription of MMP-9	Serving as a promising antimetastatic agent against oral cancer	(67)

MAPK, mitogen-activated protein kinase; EGCG, epigallocatechin-3-gallate; BTG2, BTG family member 2; ERK, extracellular signal-regulated kinase; OSCC, oral squamous cell carcinoma; EGF, epidermal growth factor; EGFR, epidermal growth factor receptor; GALR1, galanin Receptor 1; SAC, S-allylcysteine; E-cadherin, epithelial cadherin; SLUG, snail family transcriptional repressor 2; JNK, c-Jun N-terminal kinase; NF-κB, nuclear factor-κB; PI3K, phosphoinositide 3-kinase; Akt, protein kinase B; SSZ, sulfasalazine; IgG, immunoglobulin G; MT1, membrane type 1; MMP, matrix metalloproteinase; PEITC, phenethyl isothiocyanate; CTXIII, Cardiotoxin III; TPA, 12-O-tetradecanoylphorbol-13-acetate.

Thirdly, delivering inhibitors to specific cancer cells is difficult in certain cases (74), and regulating the MAPK signaling pathway will have direct or indirect impact on the expression of multiple downstream targets. Because the MAPK signaling pathway may cross-talk with other pathways, including the PI3K/Akt pathway, and because these other pathways are crucial for multiple aspects of cellular growth and differentiation (for example, epithelial mesenchymal transition) (75,76), inhibiting the MAPK signaling pathway may result in dysregulation of the components of other pathways. The cross regulation that exists between the pathways has partially been revealed by the control of Raf activity. As aforementioned, the activity of Raf is regulated by mutations of Ras in the MAPK signaling pathway, and/or by mutations of genes in in the PI3K/Akt pathway (including PI3K, phosphatase and tensin homolog and Akt), which may synergistically lead to abnormal activation of the MAPK pathway in oral cancer. This discovery indicates the presence of an intimate link between the MAPK and the PI3K/Akt pathways. In addition, these two pathways were reported to not only cause phosphorylation of downstream targets, but also to regulate a variety of pathways, including JAK/STAT, NF-κB and TGF-β pathways, via ERK and Akt phosphorylation (76). In this regard, complete exploration of these pathways and their interactions should be considered in order to improve oral cancer treatment.

In addition, current clinical practice for oral cancer often discontinues a given therapy following cancer progression. However, continuous clinical practice, including use of a certain inhibitor, is desirable in certain cases because an inhibitor of one component in the MAPK signaling pathway (for example, Ras) may partially suppress another component (for example, Raf) in the same pathway, which will contribute to improved oral cancer treatment. For instance, it is helpful to add a Ras inhibitor while keeping the patient on the Raf inhibitor with which they were already being treated. This was partially confirmed when the clinical benefits of continued ERK1/2 sub-pathway inhibition following cancer progression were demonstrated (77). However, several preclinical models revealed that discontinuation of a drug based on the inhibitors or intermittent scheduling may also slow tumor growth (78). Therefore, it is necessary to carry out further clinical research to elucidate whether continuous or intermittent dosing is optimal. Proper clinical design and scheduling are important and will enable further development of effective therapies for oral cancer patients.

Finally, inhibitors that target the MAPK signaling pathway may be cytostatic and not cytotoxic (75). There would be substantial toxicity problems for cytotoxic inhibitors. To address these problems, the inhibitors should be combined with cytotoxic chemotherapeutic drugs or radiation therapy, as well as other techniques which inhibit the growth of oral cancer (75,79). Such combinations are expected to improve oral cancer treatment.

In brief, the MAPK signaling pathway is complex due to the existence of multiple components, subfamilies and sub-pathways, which regulate different cellular functions, and to its interactions with other pathways which may elicit synergetic effects on normal and malignant cell growth. By further exploring the components, subfamilies and sub-pathways of the MAPK signaling pathway, as well as its interactions with other pathways and clinical practice modes, it will be possible to develop improved oral cancer treatments.

6. Conclusions

The present review detailed the important function of the MAPK signaling pathway in oral cancer, due to its involvement in tumor cell proliferation, differentiation, apoptosis, angiogenesis, invasion and metastasis. Based on the analysis of the individual sub-pathways and their mechanisms of action, the potential for regulation of the MAPK signaling pathway for targeted oral cancer therapy was reviewed. The challenges faced by the field and future research directions were also expanded upon. Further elucidation of the molecular components of the pathway, subfamilies, sub-pathways, interactions with other pathways and clinical practice modes will enable the development of improved oral cancer treatments.

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