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Review article

Review of the role of basic fibroblast growth factor in dental tissue-derived mesenchymal stem cells

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Background: Basic fibroblast growth factor (bFGF) plays a crucial role in various biological processes, including cell growth, survival, migration, and differentiation. In stem cell biology, bFGF is employed to maintain stemness and regulate differentiation.
Objectives: To review the role of bFGF in the behavior of stem cells, focusing particularly on human dental tissue-derived mesenchymal stem cells (DMSCs).
Methods: The articles from January 1, 1990 to March 25, 2015 in the PubMed database were searched with assigned key words (dental stem cells and (bFGF or FGF2)). Titles and abstracts of the retrieved articles were evaluated to identify inclusion criteria.
Results: Sixty-five articles were identified from the PubMed database using the assigned keywords. Eighteen articles met the inclusion criteria including: (1) articles published in English, (2) articles describing the effects of endogenous and exogenous bFGF in cell culture and animal studies, and (3) the cell model used in the study was derived from dental-related tissues, and were employed as the main articles discussed in the present narrative review.
Conclusion: bFGF supplementation enhances stem cell marker expression in DMSCs. However, the role of bFGF on osteogenic differentiation by DMSCs remains controversial.

Keywords: Basic fibroblast growth factor, dental tissue-derived stem cells, differentiation, stemness

Abbreviations

Akt = protein kinase B
ALP = alkaline phosphatase
Ank = a 12-membrane spanning protein associated with progressive ankylosing mineralization
bFGF = basic fibroblast growth factor = fibroblast growth factor 2 (FGF2)
BMP = bone morphogenetic protein
Caspase = cysteine-aspartic acid protease
CDC2 = phosphorylated cell division cycle protein 2 homolog
CDK = cyclin-dependent kinase
DAG = diacylglycerol
DMSCs = dental tissue-derived mesenchymal stem cells
DPSCs = dental pulp stem cells
Dusp6 = dual specificity phosphatase 6
EMT = epithelial-to-mesenchymal transition
ERK = extracellular signal-regulated kinase = mitogen-activated protein kinase (MAPK)
ES cells = embryonic stem cells
FGF2 = fibroblast growth factor 2 = basic fibroblast growth factor (bFGF)
FGFR = fibroblast growth factor receptor
FRS2 = fibroblast growth factor receptor substrate 2
G2/M = end of G2/entry into mitosis
Gab1 = Grb2-associated-binding protein 1
GLUT = glucose transporter
GPDH = glycerol 3-phosphate dehydrogenase
Grb2 = growth factor receptor-bound protein 2

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HDPCs = human dental pulp cells
HSPGs, heparin sulphate proteoglycans
iPS cells = induced pluripotent stem cells
IP3 = inositol-1,4,5-triphosphate
JAK = Janus kinase
JNK = c-Jun N-terminal kinases
MAPK = mitogen-activated protein kinase = extracellular signal-regulated kinase (ERK)
MEK = mitogen-activated protein kinase
MKP-3 = mitogen-activated protein kinase phosphatase-3
MSCs = mesenchymal stem cells
NF-kB = nuclear factor k-light-chain-enhancer of activated B cells
Oct4 = octamer-binding transcription factor 4
P = phosphorylation
p21 = cyclin-dependent kinase inhibitor
PC-1 = pyrophosphate-generating enzyme
PCP = noncanonical planar cell polarity
PDLSCs = periodontal ligament stem/progenitor cells
PI-3 kinase = phosphatidylinositol-4,5-bisphosphate 3-kinase
PIP2 = phosphatidyl-inositol-4,5-diphosphate
PKC = protein kinase C
PLGA = poly(lactic-co-glycolactic acid)
PLC = phospholipase C
PPARg2 = peroxisome proliferator-activated receptor-g2
Raf1 = RAF proto-oncogene serine/threonine-protein kinase
SCAPs = stem cells from the apical papilla
SH2 = Src homology 2
SHEDs = stem cells isolated from human exfoliated deciduous teeth
Shc = Src homology
SOS = Son of Sevenless
Spred = sprouty-related, EVH1 domain-containing protein
Spry = sprouty protein
SSEA-4 = stage-specific embryonic antigen 4
STAT1 = signal transducer and activator of transcription-1
XFLRT3 = (Xenopus) fibronectin leucine rich transmembrane protein 3

Basic fibroblast growth factor (bFGF) or fibroblast growth factor 2 (FGF2), a member of the fibroblast growth factor family, regulates cell growth, differentiation, migration, and survival during development and regeneration [1-3]. bFGF has various roles in stem cell biology including the maintenance of stemness and control of differentiation [4, 5]. To maintain stemness, bFGF regulates the self-renewing ability of several cell types [6]. bFGF signaling plays a crucial role in the self-renewal capacity of human embryonic stem cells (ES) and human induced pluripotent stem cells (iPS) [7]. Exogenous bFGF enhances the expression of pluripotent markers [8]. We have shown that exogenous bFGF stimulates colony-forming units and enhances the mRNA expression of pluripotent stem cell markers in stem cells isolated from human exfoliated deciduous teeth (SHEDs) and human dental pulp stem cells (DPSCs) [9, 10]. The role of bFGF in stem cell differentiation is controversial. We reported an inhibitory effect of bFGF on osteogenic differentiation of MSCs [9, 11], while others reported an inductive effect [12, 13]. Similarly, the effects of bFGF on adipogenic differentiation are controversial. These contradictory results may be a consequence of different cell types, concentrations, exposure times, and culture conditions.

DMSCs have been introduced as a stem cell source because of their accessibility and availability. MSCs can be isolated from various dental-related tissues, including dental pulp, periodontal ligaments, apical papilla, and dental follicles [14]. The isolated cells exhibit the stem cell characteristics, including the expression of mesenchymal stem cell markers and multipotential differentiation ability [14-16]. Although these cells share common characters, the DMSCs from different sources exhibit dissimilar characteristics and potency [17-20]. Various studies have examined the effect of bFGF on the behavior of these DMSCs. The results are varied. In the present article, the influence of bFGF on DMSCs is reviewed and discussed in terms of both stemness maintenance and cell differentiation.

Methods

The articles from January 1, 1990 to March 25, 2015 in PubMed database were searched using keywords. The keywords used in the search were (“1990/01/01”[Publication Date]:”2015/03/25"[Publication Date]) AND (dental stem cells and (bFGF or FGF2)). The title and abstract of retrieved articles were evaluated for inclusion in the review. The inclusion criteria were as follows: (1) articles published in English, (2) articles describing the effects of endogenous and exogenous bFGF in cell culture and animal studies, and (3) the cell model used in the study was derived from dental-related tissues.

Results

Sixty-five articles were identified from the PubMed database using the assigned keywords. Eighteen articles met with the inclusion criteria and were employed as the main articles discussed in the present narrative review.

bFGF and receptors

bFGF is a b-sheet protein that consists of 140 amino acids [21]. It contains two receptor binding sites, locating at residues 13–30 and 106–129 [22-24]. These residues bind to fibroblast growth factor receptors (FGFRs) on the cell surface [25]. FGFRs consist of four subtypes: FGFR1, FGFR2, FGFR3, and FGFR4 [26, 27]. The preferential binding ability of bFGF to its receptors may lead to a differential cell response [25]. The levels and types of receptor expression are crucial factors regulating bFGF signaling. FGFR expression levels are altered during cell proliferation or differentiation. For example, actively proliferating cells express higher FGFR than the confluent cells, implying an influence of bFGF on cell proliferation [28]. Moreover, FGFR levels are shown to increase or decrease during cell differentiation depending on cell type [29, 30]. The binding of bFGF to its receptors results in the activation of tyrosine kinase [31] and, subsequently, leads to initiation of various intracellular signaling, including phospholipase C (PLC)-g, protein kinase C (PKC), Ras (small GTPase)-mitogen-activated protein kinase (MAPK), phosphatidylinositol-4,5-bisphosphate 3-kinase (PI-3 kinase)/protein kinase B (AKT), signal transducer and activator of transcription-1 (STAT1)/ cyclin-dependent kinase inhibitor (p21), Src homology (Shc), and Src pathways [32, 33]. Further, bFGF can bind heparin and bFGF signaling can be enhanced in the presence of heparin sulfate proteoglycan [25, 34]. Heparin sulphate promotes the stability of receptor dimerization and prevents aggregation of bFGF [25, 34, 35]. A diagram summarizing the intracellular signaling induced by bFGF is shown as **Figure 1**.

bFGF and stemness maintenance

bFGF has crucial role in maintaining the stemness properties of human embryonic stem (ES) and induced pluripotent stem (iPS) cells. In general, bFGF is used as a supplemental growth factor in the culture medium of these cells to maintain them in an undifferentiated state [36, 37]. bFGF supplementation is able to maintain pluripotent marker expression in long-term culture of human ES cells [38]. Further, human ES cells cultured in bFGF supplemented serum-free culture medium are able to proliferate and maintain an undifferentiated state [7]. Correspondingly, human ES cells can be maintained in an undifferentiated state in coculture with bFGF expressing human feeder cells [37, 39-41]. However, the effect of bFGF on several characteristics of human ES and iPS cells is dose- and cell line-dependent [42].

bFGF is required to maintain the expression of the stemness markers octamer-binding transcription factor 4 (Oct4) and the transcription factor, Nanog, by human ES cells [43, 44]. Mechanistically, bFGF maintains human ES cells in an undifferentiated state via the extracellular signal-regulated kinase (ERK)1/ 2-c-Fos/c-Jun signaling pathway [39, 45]. bFGF directly regulates Nanaog expression via the ERK–mitogen-activated protein kinase (MEK) pathway [35, 44]. Supplementation with ERK inhibitor suppresses bFGF-induced Nanog expression in human ES cells [35, 44]. Moreover, bFGF represses bone morphogenetic protein (BMP) signaling in human ES cells, resulting in attenuation of differentiation and promotion of self-renewal [37]. A combination treatment of bFGF and noggin (a BMP antagonist) can sustain the undifferentiated state of human ES cells in a feeder free culture [46]. Further, bFGF inhibits human iPS and ES cell apoptosis by the inhibition of activation of cysteine-aspartic acid protease (Caspase)-3 through the ERK/serine/ threonine-specific Akt signaling pathway, indirectly preventing differentiation [47].

bFGF is involved in the self-renewal and maintenance of the multipotential differentiation ability of mesenchymal stem cells (MSCs) [4, 5]. Exogenous bFGF supplementation or endogenous bFGF overexpression enhances proliferation of human MSC [48, 49]. Addition of exogenous bFGF does not alter the multipotential differentiative ability of these stem cells [49]. CyclinD1, cyclinD3, cyclin-dependent kinase (CDK)-4, and phosphorylated cell division cycle protein 2 homolog (CDC2) protein expression are dramatically upregulated in bFGF-treated human MSCs, resulting in enhancement of proliferation [50]. Moreover, bFGF promoted the mRNA expression of pluripotent stem cell markers in MSC isolated from various tissues [9, 10, 50]. Together, these results suggest an important role for bFGF in controlling the stemness of both human pluripotent and multipotent stem cells.

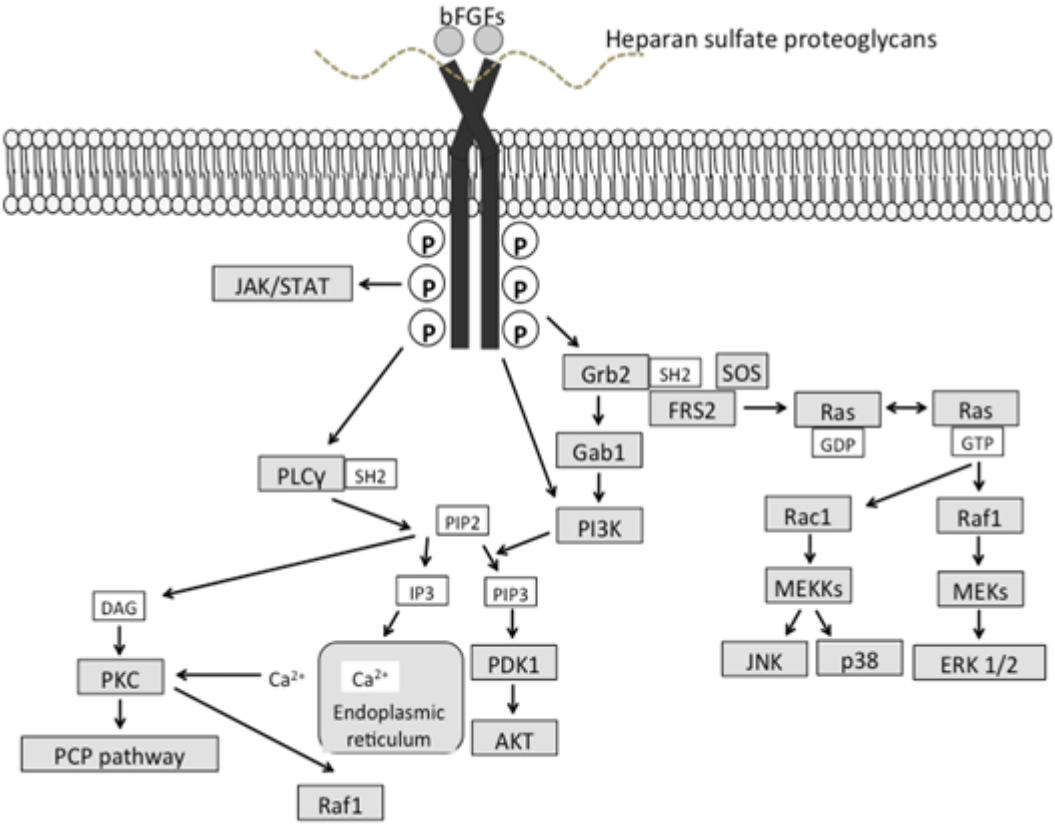

Figure 1. bFGF intracellular signaling. Activation by receptor autophosphorylation triggers diverse signaling cascades, including the Ras/ mitogen-activated protein kinase (MAPK), phosphatidylinositol-4,5-bisphosphate 3-kinase (PI-3 kinase)/protein kinase B (Akt), phospholipase C (PLC)-g/Ca2 and the Janus kinase (JAK)/signal transducer and activator of transcription (STAT) pathways. Phosphorylation of the docking protein, fibroblast growth factor receptor substrate 2 (FRS2) is followed by growth factor receptor-bound protein 2 (Grb2) activation, which in turns activates either the Ras/MAPK cascade via Son of Sevenless (SOS), or the PI-3 kinase/Akt pathway via Grb2-associated-binding protein 1 (Gab1). PI-3 kinase can also be activated directly by tyrosine phosphorylation or alternatively by Ras1. The other main transduction pathway involves PLC. The Src homology 2 (SH2) domain of the PLC interacts directly with the receptor leading to the hydrolysis of phosphatidyl-inositol-4,5-diphosphate (PIP2) to inositol-1,4,5-triphophate (IP-3) and diacylglycerol (DAG). Inositol-1,4,5-triphosphate (IP-3) releases Ca^{2+} from the endoplasmic reticulum (ER), while DAG activates protein kinase C (PKC) that in turn can activate the noncanonical planar cell polarity (PCP) pathway and RAF proto-oncogene serine/threonine-protein kinase (Raf1). Feedback inhibitors such as dual specificity phosphatase 6 (Dusp6)/mitogen-activated protein kinase phosphatase-3 (MKP-3), sprouty protein (Spry), FRS2a, sprouty-related, EVH1 domain-containing protein (Spred), and Sef involved in signal attenuation, and enhancers such as fibronectin leucine rich transmembrane protein 3 (XFLRT3) can also contribute to the overall levels of bFGF signaling. HSPGs, heparin sulphate proteoglycans; EMT, epithelial-to-mesenchymal transition; P, phosphorylation (modified from Villegas et al., 2010) [110] □ 2010 Wiley-Liss, Inc. with permission for reuse.

bFGF and cell differentiation

Addition of exogenous bFGF effects stem cell differentiation toward various lineages, including osteogenic, adipogenic, chondrogenic, myogenic, and neurogenic lineages. The present review focuses briefly on the osteogenic, adipogenic, and neurogenic lineages.

bFGF and osteogenic differentiation

The influence of bFGF on osteogenic differentiation is controversial. bFGF both positively and negatively regulates osteogenic differentiation. *Fgf2*-knockout mice exhibit reduced bone mass and osteogenic differentiation [51]. Correspondingly, bFGF promotes cell proliferation and enhanced osteogenic

differentiation in bone marrow stromal cells [52]. bFGF treatment upregulates alkaline phosphatase (ALP) activity, osteocalcin mRNA expression, calcium deposition, and bone nodule formation in vitro [53, 54]. Moreover, bFGF and BMP-2 in combination synergistically induce osteogenic potency in rat bone marrow MSCs [55]. The delivery of bFGF using various approaches (i.e. coral scaffold and collagen hydrogel) results in the promotion of osteogenic differentiation in human bone marrow MSCs [56-58]. However, the selective response of the osteogenic marker gene to bFGF, and the specific time point in which bFGF could promote osteogenic differentiation, suggested treatment with bFGF enhanced osteopontin, but decreased type I collagen expression [59]. Moreover, bFGF exposure at an early stage of differentiation promotes osteogenic differentiation, while inhibiting it in later stages [60].

Besides the positive regulation of osteogenic differentiation by bFGF, some contradictory reports are noted [9, 11, 61]. A combination of BMP-2 and bFGF inhibits the inductive effect of BMP-2 in a rat femur defect model of bone formation [62]. Further, supplementation with bFGF alone could strongly attenuate osteogenic differentiation in MSC derived from various tissue sources [9, 11, 63]. bFGF might alter a specific process during osteogenic differentiation. For example, exogenous bFGF reduces the ALP activity in osteogenic medium, but does not affect mineralization [64]. In mouse preosteoblasts, bFGF inhibits mineralization, possibly via the upregulation of pyrophosphate-generating enzyme (PC-1) and Ank, a 12-membrane spanning protein associated with progressive ankylosing mineralization (e.g. chondrocalcinosis and craniometaphyseal dysplasia), and downregulation of tissue nonspecific ALP. This bFGF-induced gene expression alteration results in the accumulation of a potent inhibitor of mineralization, pyrophosphate, leading to attenuation of mineralization [65, 66]. The intracellular mechanism(s) require further investigation to clarify the role of bFGF in osteogenic differentiation.

bFGF and adipogenic differentiation

In bFGF knockout mice, an increase of bone marrow fat accumulation assessing by osmium tetroxide labeling and Oil Red O staining was noted in vivo [67]. Further, addition of exogenous bFGF can decrease intracellular lipid accumulation in bone marrow-derived cells in bFGF knockout mice [67]. Correspondingly, bFGF overexpression in human MSC results in a slight decrease of adipogenic marker expression, and attenuates intracellular lipid accumulation of cells cultured in an adipogenic induction medium [68]. Together, these results imply a suppressive effect of bFGF on adipogenic differentiation. Further, bFGF suppressed adipogenic differentiation via extracellular signal-regulated kinase 1 and 2 (ERK1/2) activation [69].

By contrast, bFGF enhances adipocyte differentiation in human embryonic stem cell-derived MSCs [70]. Upregulation of peroxisome proliferator-activated receptor-g2 (PPARg2) by MSC is observed upon bFGF supplementation in an adipogenic induction medium [71]. Moreover, bFGF binding to heparinized decellularized adipose tissues promotes adipose tissue formation in vivo [72]. In addition, bFGF supplementation upon adipogenic differentiation of human adipose-derive stem cells can enhance the upregulation of PPAR g2 and glucose transporter (GLUT) type 4 mRNA expression, lipid accumulation, and glycerol 3-phosphate dehydrogenase (GPDH) activity in vitro [73]. An adipogenic enhancing effect was shown in a 3D culture system employing a poly(lactic-co-glycolactic acid) (PLGA) scaffold. Adipogenesis of bone marrow MSCs is enhanced upon bFGF supplementation [74]. The apparently contradictory effects of bFGF on adipogenic differentiation are noted to be similar to those on osteogenic differentiation [67]. Thus, further investigation is required to determine the role of bFGF in adipogenic differentiation in specific cell types.

bFGF and neurogenic differentiation

bFGF promotes neuronal differentiation. bFGF induces cell division and neuronal differentiation by chromaffin cells, olfactory neuroblastoma cells, amniotic epithelial cells, and spinal cord neurons [75-77]. In MSCs, bFGF and neurotrophin 3 supplementation promoted neuronal differentiation [78]. Further, bFGF promotes the mRNA expression of neuronal markers in various cells, including bone marrow derived MSCs, muscle-derived stem cells, DPSCs, and adipose stem cells [79-82]. For example, the addition of bFGF alone in neurobasal medium was sufficient to enhance neuronal differentiation of dental pulp stem cells (DPSCs), as determined by the expression of b3-tubulin [9]. Moreover, bFGF is indispensable for Schwann cell induction from bone marrow MSCs and this process is regulated via the MAPK/ERK signaling pathway [83].

bFGF and other cell differentiation

Beside the lineages discussed above, there are several reports of the influence of bFGF on cell differentiation potency toward other specific cell lineages i.e. epithelial, chondrogenic, and myogenic lineages. Exemplified by bFGF treatment of lens-epithelial cells promotes cell proliferation and lens-fiber differentiation [84]. bFGF enhances mature cardiomyocyte differentiation from cardiac precursor cells and mouse embryonic stem cells [85, 86]. Further, bFGF priming or bFGF immobilized on biomaterials can promote genotypic and phenotypic changes of MSCs toward fibroblasts [87, 88].

bFGF in DMSCs

bFGF enhances stemness in many types of DMSCs. The bFGF enhances the expression of embryonic stem cell markers (Oct4, Rex1, and Nanog) in DPSCs [9]. Moreover, an increase in the number of cells recognized by the STRO monoclonal antibody (STRO-1^+ cells) is observed in bFGF treated DPSCs and human periodontal ligament stem/progenitor cells (PDLSCs) [89, 90]. Supplementation of medium in both short- and long-term cultures with bFGF leads to an increase in mRNA expression of pluripotent markers in SHEDs [10]. Similarly, bFGF enhances stem cell marker expression in stem cells from the apical papilla (SCAPs) [91]. An increase of colony forming unit ability is observed when SHEDs from normal and inflamed pulp tissues are supplemented with exogenous bFGF [10, 92]. Exogenous bFGF does not influence the proliferative ability of SHEDs [10, 61]. By contrast, bFGF enhanced the proliferation of DPSCs, PDLSCs, and SCAPs [89, 91, 93-95]. End of G2/entry into mitosis (G2/M) is upregulated when PDLSCs are treated with bFGF [95]. bFGF induces proliferation of human dental pulp cells (HDPCs) and tends to enhance stem cell surface marker proteins STRO-1 and stage-specific embryonic antigen 4 (SSEA-4) [96].

Osteogenic differentiation is attenuated in the presence of exogenous bFGF in osteogenic culture medium. Attenuation of ALP enzymatic activity, osteogenic marker expression, and mineralization is noted in SHEDs, DPSCs, SCAPs, and PDLSCs [9, 11, 60, 91, 93, 97]. Correspondingly, FGFR inhibitor supplementation promotes ALP activity and mineralization by SHEDs in vitro [98]. bFGF possibly inhibits the Wnt/□-catenin signal transduction pathway, which has been shown in SHEDs [61]. Transplantation with PDLSCs and bFGF results in a decrease of bone formation in mice [93].

By contrast, exogenous bFGF enhances ALP activity, mineralization, and odontoblastic marker gene expression in primary HDPCs [96]. bFGF treatment of HDPCs induces chemokine mRNA expression via MAPKs (ERK1/2, p38, c-Jun N-terminal kinases (JNK)), nuclear factor k-light-chain-enhancer of activated B (NF-kB) cells, and PKC pathways [96]. bFGF pretreatment for 1 week before osteogenic induction enhances osteogenic differentiation ability in vitro and in vivo [60]. BMP-2 and bFGF promote the formation of new bone [99]. Gelatin carriers releasing human recombinant bFGF induce periodontal regeneration in artificially created furcation class II bone defects in beagle dogs [97] and primates [100]. The effects of bFGF in DMSCs remain controversial. A summary of the in vitro and in vivo effects of bFGF in dental-derived stem/progenitor cells is shown in **Tables 1 and 2**.

Preclinical study of the use of bFGF in dentistry

bFGF-loaded hydrogel enhances revascularization and pulp-like tissue regeneration in human endodontic treated teeth implanted subcutaneously in mice [101]. bFGF-releasing scaffolds promoted robust dentin formation in a rat model of molar defect [102]. Controlled bFGF release results in localized dentin formation in the defect area [102, 103]. The dose administered is a critical factor for the dentin formation. A low dose (0.05 mg/ml) fails to promote dentin regeneration, while a high dose (5 mg/ml) results in scattered and incomplete dentin formation [103]. Correspondingly, bFGF (dose 30 ng) did not promote dentin bridge formation, but instead fibrous formation with some inflammation [104].

bFGF promotes periodontal tissue regeneration in canine periodontal defects [97, 105, 106]. This regeneration may be the result of the proliferative effect of bFGF on periodontal ligament cells as demonstrated in vitro [107]. A positive effect of bFGF is observed in a canine model of alveolar bone regeneration [108, 109].

Conclusion

The regulation of stem cells behaviors by bFGF may depend on several factors, including dose, exposure time, and cell type. The influence of bFGF on differentiation is controversial. Careful investigations of bFGF function in specified cell types in specific settings are necessary to understand the complex regulation of dental MSC behaviors by bFGF.

Table 1. In vitro effects of basic fibroblast growth factor (bFGF)

Cell type	In vivo results	References
DPSCs	(+) cell migration	Nishino et al., 2011 [111]
	(+) cell proliferation	Morito et al., 2009 [89] Lee et al., 2015 [112] He et al., 2008 [94]
	(+) colony forming unit	Osathanon et al., 2011 [9]
	(+) matrix deposition and cell viability	Yang et al., 2015 [113]
	(+) stem cell marker expression (STRO-1, Oct4, Nanog, Rex1)	Morito et al., 2009 [89] Osathanon et al., 2011 [9]
	(–) osteoblast differentiation	Qian J et al., 2014 [60] Morito et al., 2009 [89] Osathanon et al., 2011 [9]
	(+) osteoblast differentiation (6 day or 2 weeks bFGF priming)	Qian J et al., 2014 [60] Lee et al., 2015 [112]
	(+) neurogenic differentiation	Sasaki et al., 2008 [114] Osathanon et al., 2011 [9]
PDLSCs	(+) cell proliferation	Kono et al., 2013 [95] Lee et al., 2012 [93] Lee et al., 2015 [112] Takeuchi et al., 2015 [115]
	(–) c-Kit expression	Suphanantachat et al., 2014 [116]
	(–) osteoblast differentiation	Lee et al., 2012 [93] Osathanon et al., 2013 [11]
SHEDs	(+) colony forming unit	Nowwarote et al., 2015 [98] Sukarawan et al., 2014 [10] Osathanon et al., 2013 [11] Kim et al., 2014 [92]
	No influence on cell proliferation	Li et al., 2012 [61] Sukarawan et al., 2014 [10]
	(+) stem cell marker expression (Oct4, Nanog, Rex1)	Sukarawan et al., 2014 [10]
	(–) osteoblast differentiation	Nowwarote et al., 2015 [98] Osathanon et al., 2013 [11] Kim et al., 2014 [92] Li et al., 2012 [61]
	(+) adipogenic and chondrogenic differentiation	Kim et al., 2014 [92]
SCAPs	(+) cell proliferation and colony forming unit (+) stem cell marker expression (Oct4, Nanog, Rex1, Sox2, STRO-1)(–) osteoblast differentiation	Wu et al., 2012 [91]
Dental pulp cells	(+) cell migration	Takeuchi et al., 2015 [115]
	(+) cell proliferation	Takeuchi et al., 2015 [115] Kim et al., 2010 [96]
	(–) osteoblast differentiation	Takeuchi et al., 2015 [115]
	(+) odontoblast differentiation	Kim et al., 2010 [96]
Periodontal ligament cells	(+) cell migration and cell proliferation	Takeuchi et al., 2015 [115] Kono et al., 2013[95]
	(+) proliferation of STRO-1^{+}/$CD146^{+}$ cells	Hidaka et al., 2012 [90]
	(–) osteogenic differentiation	Dangaria et al., 2009 [117]
Dental follicle cells	(–) osteogenic differentiation	Dangaria et al., 2009 [117]

DPSCs = dental pulp stem cells; periodontal ligament stem/progenitor cells (PDLSCs) SHEDs = stem cells isolated from human exfoliated deciduous teeth; stem cells from the apical papilla (SCAPs) octamer-binding transcription factor 4 (Oct4)

Table 2. In vivo effects of basic fibroblast growth factor (bFGF)

Cell types	In vivo results	References
PDLSCs	(–) bone formation in subcutaneous implantation	Lee et al., 2012 [93]
DPSCs	(–) bone formation (1 week bFGF priming)	Qian et al., 2014 [60]
	(+) bone formation (2 week bFGF priming)	Yang et al., 2015 [113]
	(+) revascularization and cell migration in an ectopic tooth slice transplantation model	
SHEDs	(+) dentin-like structure formation in an ectopic transplantation models	Kim et al., 2014 [92]
	(–) bone formation in ectopic transplantation models	Li et al., 2012 [61]
Primary dental pulp cells from deciduous teeth (in vivo delivery without cell incorporation)	(+) wound healing in a murine full-thickness skin defect model	Nishino et al., 2011 [111]
	(+) revascularization, recellularization, and odontoblastic differentiation in an ectopic tooth transplantation model	Takeuchi et al., 2015 [115] Suzuki et al., 2011 [101]

DPSCs = dental pulp stem cells; PDLSCs = periodontal ligament stem/progenitor cells; SHEDs = stem cells isolated from human exfoliated deciduous teeth.

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Conflict of interest statement

The authors have no conflicts of interest to declare.

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