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Osteoclast Stimulatory Transmembrane Protein (OC-STAMP): A Key Regulator in Osteoclast Fusion

Mayyadah Abdullah Alkuwayti

Department of Biological Sciences, College of Science, King Faisal University, Al Ahsa, Saudi Arabia

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ABSTRACT

The integrity of bone mass depends on the balance between osteoblastic bone formation and osteoclastic bone resorption. Osteoclasts derived from hematopoietic stem cells in bone marrow via a mechanism mediated mainly by two cytokines: the receptor activator of nuclear factor-ΚB ligand (RANKL) and macrophage colony-stimulating factor (M-CSF). Osteoclast stimulatory transmembrane protein (OC-STAMP) is a newly identified master fusogen that functions as a regulator of osteoclastogenesis. It is considered a key factor during macrophage fusion and differentiation. Osteoclast formation is mediated by a variety of factors and mechanisms other than OC-STAMP. Furthermore, numerous preclinical studies have investigated drugs that could target osteoclast differentiation, to allow better management of pathological bone metabolism. To improve understanding of osteoclast fusion and differentiation processes, this review will discuss recent findings on OC-STAMP and related signalling pathways.

KEYWORDS

Osteoblasts, osteoclastogenesis, RANKL, differentiation, DC-STAMP, osteoclast fusion molecules

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1. Introduction

Bone remodelling is a metabolic process that plays a critical role in bone homeostasis. The remodelling of bone is controlled by the interactions of three cell types: bone synthesising cells (osteoblasts), bone degrading cells (osteoclasts) and mechanosensing cells (osteocytes) that originate from osteoblasts and are embedded within the bone matrix (Chambers, 2000; Kylmaoja *et al.*, 2016; Parfitt, 2002). An imbalance between bone formation and resorption can lead to several bone diseases including osteoporosis, rheumatoid arthritis, multiple myeloma, periodontal disease and metastatic cancers (Boyle *et al.*, 2003).

Bone production is carried out by osteoblasts, which are mononucleate cuboid cells. Immature mesenchymal stem cells give rise to osteoblasts. A giant Golgi apparatus and an abundance of rough endoplasmic reticulum appear when they are active (Caetano-Lopes *et al.*, 2007). During bone development, osteoblasts form the components of bone matrix and regulate osteoclasts and the deposition of minerals (Florencio-Silva *et al.*, 2015).

Osteoclasts are multinucleated cells derived from monocyte/ macrophage precursor cells (Teitelbaum and Ross, 2003). Osteoclasts are characterised by bone resorption properties and are uniquely capable of forming a ruffled membrane during contact with bone, allowing the cells to absorb the bone matrix (Blair *et al.*, 1989; Boyce, 2013).

Osteoclast fusion is considered one step in osteoclastogenesis, a complicated process consisting of several stages: differentiation of the progenitor, mononuclear pre-osteoclasts, fusion to form multinucleated mature osteoclasts and activation of these osteoclasts to resorb bones (Xing *et al.*, 2012). Several studies have demonstrated that osteoclast stimulatory transmembrane protein (OC-STAMP) plays a crucial role in osteoclast fusion (Ishii *et al.*, 2018; Miyamoto *et al.*, 2012; Witwicka *et al.*, 2015).

OC-STAMP is a multi-pass transmembrane protein with six-transmembrane helical domains, consisting of 498-amino acids with a putative glycosylation site known to be important for protein stability. Both its N- and C-termini face the cytosol (Witwicka *et al.*, 2015).

It has been shown that OC-STAMP is essential for macrophage differentiation and polarisation (Yuan *et al.*, 2017). The role of OC-STAMP during cell differentiation was first demonstrated in an in vivo study of osteoclastogenesis by Yang *et al.* in 2008. Further investigations conducted by Miyamoto *et al.* (2012) identified the involvement of OC-STAMP in osteoclast fusion. Most tissues express OC-STAMP, which suggests it also has functions in other tissues (Kim *et al.*, 2011).

Dendritic cell-specific transmembrane protein (DC-STAMP) is also considered an important regulator of osteoclastogenesis (Zhang *et al.*, 2014). OC-STAMP and DC-STAMP share several common features in terms of their structure and function. For example, both are transmembrane proteins, play an important role in osteoclast fusion and are stimulated by the receptor activator of osteoclast fusion, RANKL. (Chiu and Ritchlin, 2016). This paper reviews the current knowledge of OC-STAMP, focusing primarily on its regulation of osteoclast fusion, its interaction with other signalling pathways and its structure.

2. The Mechanism of Osteoclast Formation and Fusion

During embryogenesis, the early stages of bone formation involve cellular fusion. Remodelling of bone depends on bone resorption by multinucleated cells (osteoclasts). Therefore, osteoclast cell–cell fusion is a key process during osteoclastogenesis to guarantee effective bone resorption.

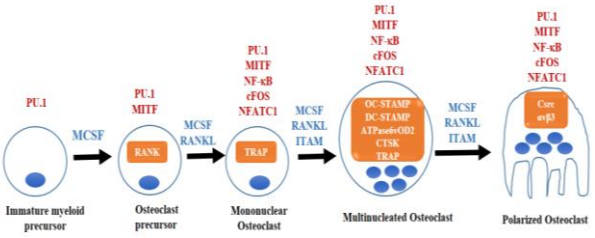
The mononuclear precursor osteoclast cells differentiate into mature multinucleate osteoclasts due to activation by two cytokines: RANKL and M-CSF, also known as colony stimulating factor 1 (CSF-1). Osteoclast differentiation occurs via multiple rounds of cell fusion (Charles and Aliprantis, 2014). The proliferation and survival of osteoclast progenitor cells depends on M-CSF receptor activation (Dai *et al.*, 2002). RANKL is a critical regulator of osteoclastogenesis. It is a member of the tumour necrosis factor (TNF) family and

Corresponding Author: Mayyadah Abdullah Alkuwayti 00966504913032, malkwaiti@kfu.edu.sa

activates RANK (Dougall *et al.*, 1999).

The formation of osteoclasts from immature myeloid precursor cells is a complex process involving several stages. These include precursor cell proliferation, cell fusion to form multinucleated cells, cellular polarisation, attachment to bone and activation of functional resorption. These stages are controlled by many factors, specifically those affecting the two transmembrane proteins DC-STAMP and OC-STAMP, that induce cell–cell fusion by reorganising the cell membrane structure (Ishii *et al.*, 2018); see Figure 1.

Figure 1: Osteoclast formation



Immature myeloid precursor cells give rise to osteoclasts. They upregulate RANK when activated by M-CSF and afterwards generate mononuclear osteoclasts that merge into multinucleate cells when stimulated by M-CSF and RANKL. The multinucleated osteoclasts become effective bone resorbing cells when they polarise and attach to bone. The stimuli needed for osteoclast formation to progress are shown in blue on Figure 1. Transcription factors for osteoclast formation are shown in red. Critical osteoclast genes are highlighted in orange boxes. Abbreviations: PU.1, PU box-binding protein; M-CSF, macrophage colony-stimulating factor; MITF, microphthalmia-associated transcription factor; RANKL, nuclear factor kappa-B ligand-receptor activator; ITAM, immunoreceptor tyrosine-based activation motif; NF-κB, nuclear factor κB; NFATc1, nuclear factor of activated T cells; RANK, receptor-activating NFkB; OC-STAMP, osteoclast stimulatory transmembrane protein; DC-STAMP, dendritic cell-specific transmembrane protein; ATP6V0d2 ATPase, H+ transporting, lysosomal 38 kDa, V0 subunit d2; TRAP tartrate resistant acid phosphatase; CTSK, cathepsin K.

OC-STAMP knockdown suppressed the formation of multinucleated osteoclasts and reduced the expression of the cell-fusion-related protein meltrin-α (Kim *et al.*, 2011). Additionally, myeloid precursor cells from OC-STAMP-knockout mice do not form mature multinucleated osteoclasts when induced by RANKL in vitro (Miyamoto *et al.*, 2012). Continued research into aspects of OC-STAMP-knockouts provides insight into the vital role of this protein in pre-osteoclast fusion (Witwicka *et al.*, 2015).

3. OC-STAMP as a Regulator of Cellular Fusion in the Formation of Osteoclasts and Foreign Body Giant Cells

Cell–cell fusion is a regulated biological phenomenon that is necessary in mammals for various processes including fertilisation, skeletal muscle growth, placenta formation and development of the immune system. It also involves several different cell types and tissues, such as cells from the macrophage lineage including osteoclasts and foreign body giant cells (FBGCs) (Blumenthal *et al.*, 2003; Hernandez *et al.*, 1996).

Monocyte/macrophage cells originating from bone marrow can merge to generate FBGCs. This is triggered by foreign body reactions that occur at either implantation or infection sites (Miyamoto *et al.*, 2012). FBGCs also act to loosen implanted medical substances, including artificial joints and other devices. Interleukin (IL)-4 and the granulocyte–macrophage colony-stimulating factor (GMCSF) are both essential for FBGC generation (McNally and Anderson, 1995). However, IL-4 prevents osteoclasts from facilitating the differentiation of monocyte/macrophages into FBGCs (Bendixen *et al.*, 2001). GMCSF has a dual effect on osteoclasts depending on the length of exposure. Short-term osteoclast progenitor exposure to GMCSF contributes to increased osteoclast formation, whereas long-term osteoclast exposure decreases their formation (Hodge *et al.*, 2004).

Although osteoclasts and FBGCs are functionally different, they share many common features. Both are formed from the macrophage cell lineage. They are positive for tartrate-resistant acid phosphatase (TRAP) staining, which is an important osteoclast activity marker (Anazawa *et al.*, 2004; Khan *et al.*, 2013). They play a role in peri-implantitis and express the cell surface receptor proteins DC-STAMP and OC-STAMP (Yagi *et al.*, 2005; Yang *et al.*, 2008).

OC-STAMP has been identified as a multi-pass transmembrane protein that plays an important role in osteoclast formation. It has been demonstrated that OC-STAMP regulates both osteoclast fusion and differentiation (Ishii *et al.*, 2018; Miyamoto *et al.*, 2012; Witwicka *et al.*, 2015; Yang *et al.*, 2008). Its mRNA is highly upregulated in pre-osteoclast primary cell cultures and RAW264.7 cells, a cell line of macrophage/osteoclast mouse precursors, when the cells are stimulated with RANKL (Yang *et al.*, 2008). Further studies using either OC-STAMP knockdown or OC-STAMP antibody approaches continue to support the essential role of OC-STAMP in osteoclast fusion. A study conducted by Kim *et al.* (2011) identified that expression of OC-STAMP might be needed for the entire formation process of multinucleated osteoclasts. This is based on observations in RAW264.7 cells and mouse bone marrow-derived macrophages (BMMs), that RANKL induction of OC-STAMP causes OC-STAMP mRNA, to be detectable throughout osteoclastogenesis. In addition, they demonstrated that the knockdown of OC-STAMP did not affect the viability of the cell. However, OC-STAMP inhibited the bone resorption activity of osteoclasts, highlighting the involvement of OC-STAMP expression in the formation of multinucleated osteoclasts.

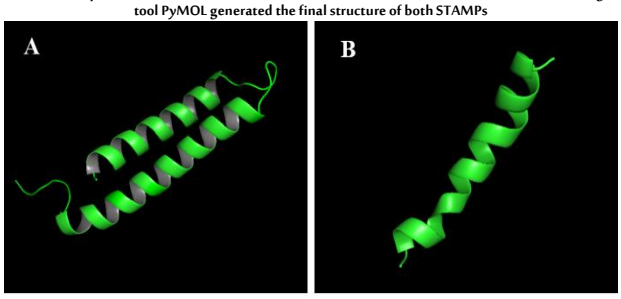
It was claimed that OC-STAMP induces cell fusion to form osteoclasts and FBGCs. The expression of OC-STAMP was shown to be upregulated with the activation of osteoclast and FBGC formation. OC-STAMP-deficiency in both cell types resulted in the complete abolition of fusion (Miyamoto *et al.*, 2012). Interestingly, OC-STAMP expression that was exogenous to either osteoclasts or FBGCs in OC-STAMP-deficient mice restored the OC-STAMP cell–cell fusion activity. Khan *et al.* (2013) have consistently indicated that inhibition of OC-STAMP by anti-OC-STAMP antibody leads to reduced fusion during osteoclast and FBGC formation. However, osteoclasts and FBGCs, in turn, exhibited increased expression of OC-STAMP compared with macrophages in this study. OC-STAMP knockout (OC-STAMP-KO) bone marrow mononuclear cells (BMMCs) reveal a total failure of osteoclast precursors to fuse, yet this deficiency was recovered by OC-STAMP expression (Witwicka *et al.*, 2015). Additionally, restoring OC-STAMP expression in OC-STAMP-KO BMMCs increased both osteoclast fusion and their resorbing activity. All the above authors have suggested that OC-STAMP plays an important role in cell fusion.

4. Transmembrane Proteins of Osteoclasts: OC-STAMP and DC-STAMP Relationship and Cooperation

DC-STAMP was initially defined as a seven-transmembrane protein expressed in dendritic cells, subsequently found in macrophages and osteoclasts and induced by IL-4 (Hartgers *et al.*, 2000; Staege *et al.*, 2001; Yagi *et al.*, 2005). DC-STAMP's role during cell fusion in

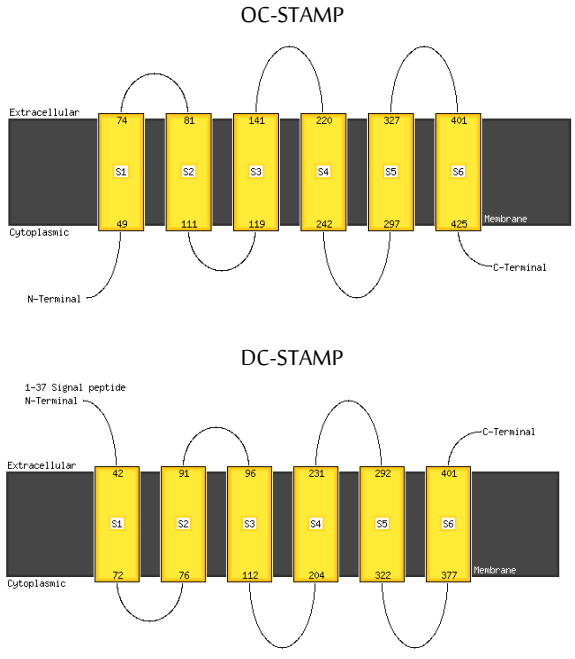
osteoclastogenesis was first demonstrated in an in-vitro study (Kukita *et al.*, 2004). Both DC-STAMP and OC-STAMP have common characteristics; for instance, based on its structure, DC-STAMP is considered to belong to the G-protein-coupled receptor (GPCR) superfamily (Mensah *et al.*, 2010). Additionally, based on its homology with DC-STAMP, OC-STAMP may also belong to the GPCR family (Witwicka *et al.*, 2015). Both are expected to be multi-pass transmembrane proteins without much direct homology between amino acids, and thus, significant similarity in the secondary structure is expected (Yang *et al.*, 2008). Furthermore, OC-STAMP possesses an amino acid residue at position193, corresponding to a DC-STAMP family consensus, whereas overall there is just 17% homology between OC- and DC-STAMP. (Yang *et al.*, 2008). According to predictions by the PHYRE 2 server, the 3D structures of the two STAMPs are different (see Figure 2).

Figure 2: The PHYRE 2 server generated the predicted 3D structure of the OC-STAMP (NP_083297) (A) and the predicted 3D structure of the DC-STAMP (NP_083698) (B). The molecular modelling tool PyMOL generated the final structure of both STAMPs



Although most transmembrane structure predictions for OC- and DC-STAMP identified six-transmembrane domains with the amino- and carboxy-termini on the cytosolic side, a seven-pass transmembrane structure for DC-STAMP was predicted in some studies (Hartgers *et al.*, 2000). In contrast, the PHYRE 2 server predicted six transmembrane helices for both OC- and DC-STAMP, with intracellular locations for both OC-STAMP termini and extracellular locations for both DC-STAMP termini. See Figure 3.

Figure 3: Predicted transmembrane helix regions of OC-STAMP and DC-STAMP by PHYRE 2



The grey shaded region represents the cell membrane. S1—S6 are the transmembrane domains. The N- and C-termini of OC-STAMP are predicted to be intracellular, while both DC-STAMP termini are predicted to be extracellular.

In addition to their structural similarity, the STAMPs have similar functions. Both OC-STAMP and DC-STAMP were shown to be involved in the fusion and differentiation of osteoclasts. RANKL induced expression of both OC- and DC-STAMP causing the concentration of both proteins to increase during the fusion process. When RANKL was suppressed by siRNA or antibody this hindered the formation of multinucleated osteoclasts, whereas the differentiation of multinucleated osteoclasts was encouraged by the overexpression of both STAMP proteins (Kukita *et al.*, 2004; Yang *et al.*, 2008). It was shown in vitro that isolated osteoclast precursor cells from each STAMP's knockout (KO) mice are unable to develop mature multinucleated osteoclasts under RANKL stimulation (Miyamoto *et al.*, 2012). It was demonstrated that both STAMPs are involved in facilitating bone lytic diseases, including periodontitis, by upregulating osteoclast fusion during osteoclastogenesis. The role of both STAMPs in periodontitis has been investigated using anti-DC-STAMP- and anti-OC-STAMP-mouse monoclonal antibodies to block the action of the STAMPs, resulting in suppression of the large multinuclear osteoclasts (TRAP+) fusion and pit formation (Ishii *et al.*, 2018; Wisitrasameewong *et al.*, 2017).

Although both STAMPs are structurally and functionally quite similar, these proteins are distinct, cannot be interchanged and are expressed independently of each other. For example, normal expression of DC-STAMP in both OC-STAMP-deficient mice osteoclasts and FBGCs was observed and vice versa. This indicates that OC- and DC-STAMP can independently regulate cell fusion in osteoclasts and FBGCs (Miyamoto *et al.*, 2012). In addition, when the OC- or DC-STAMP deficient cell cultures were mixed, the fusion conditions of osteoclast and FBGC cells were not induced. In adult mice, DC-STAMP-KO mice exhibit an osteopetrotic bone phenotype but OC-STAMP-KO mice do not. However, osteoclasts from both STAMP knockout mice had major deficiencies in resorption. Unexpectedly, OC-STAMP-KO lacked a skeletal phenotype while DC-STAMP-KO mice increased the volume of cancellous bone tissue (Miyamoto *et al.*, 2012; Yagi *et al.*, 2005). Overexpression of OC-STAMP cannot complement DC-STAMP deficiency. Correspondingly, DC-STAMP overexpression is unable to complement OC-STAMP deficiency (Chiu and Ritchlin, 2016). Therefore, it remains to be determined whether OC-STAMP and DC-STAMP can interact with each other during osteoclastogenesis.

5. Regulation of OC-STAMP Expression

Ligands for OC-STAMP have not been identified. Therefore, the entire landscape of the downstream signalling pathways remains to be discovered (Kodama and Kaito, 2020). However, it was demonstrated that RANKL significantly induces OC-STAMP mRNA expression in several tissues (Kim *et al.*, 2011).

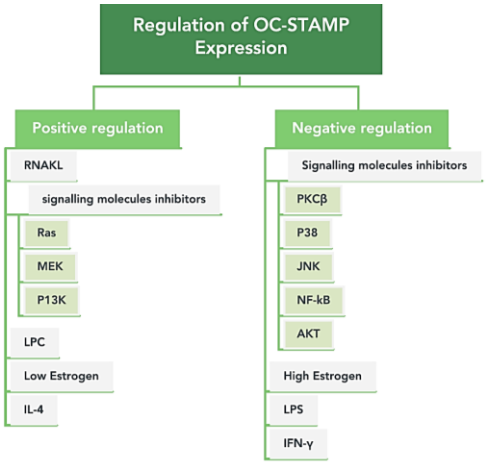
The effect of several signalling pathways associated with the initial stage of osteoclastogenesis, such as the protein kinase C β (PKCβ), p38 mitogen-activated protein kinase, c-jun N-terminal kinase (JNK) and nuclear factor kappa *B* (NF-κB) pathways, have been tested for their effect on the expression of OC-STAMP mRNA induced by RANKL. It was found that the PKCβ, p38 and NF-κB pathways can totally inhibit the expression of OC-STAMP mRNA, whereas the JNK pathway suppressed the expression of OC-STAMP mRNA only slightly. Furthermore, other inhibitory signalling pathways associated with mature osteoclast survival, such as Ras, mitogen-activated kinase kinase (MEK), phosphatidylinositol 3-kinase (PI3K) and protein kinase B (AKT), also known as PKB, can be grouped into two categories based on their impact on the expression of OC-STAMP mRNA induced by RANKL. The first group, including Ras, MEK and

PI3K, improved the expression level of OC-STAMP, whereas AKT inhibited OC-STAMP mRNA expression (Kim *et al.*, 2011); see Figure 3.

It has been demonstrated that some steroid hormones, including oestrogens, regulate osteoclastogenesis to enhance bone density and reduce bone resorption (Boyle *et al.*, 2003; Sipos *et al.*, 2009). Kim *et al.* (2011) have shown that the expression level of OC-STAMP mRNA in the ovary was lower than in other tissues, indicating it may be down-regulated by oestrogen. Further investigations have revealed a dual effect of Estradiol on OC-STAMP mRNA expression: low concentrations of 17β-Estradiol (E2) results in an upregulation of OC-STAMP expression, whereas high concentrations of E2 result in a down-regulation of OC-STAMP expression. However, continuous analysis of the action of oestrogens on the regulation of OC-STAMP remains to be carried out.

In addition, OC-STAMP is sensitive to inflammatory mediators which correlate closely with bone loss in several clinical and experimental conditions. For example, RAW264.7 cells exhibited a fourfold increase of OC-STAMP following treatment with the pro-inflammatory lipid lysophosphosphatidylcholine for two hours. Furthermore, it was observed that OC-STAMP was significantly reduced by lipopolysaccharide (LPS) and cytokine interferon- γ (IFN-γ) activation but improved by IL-4 treatment (Yang *et al.*, 2008); see Figure 4.

Figure 4: Diagram summarising the regulation of OC-STAMP expression. Positive regulatory factors improve the expression of OC-STAMP. Negative regulatory factors inhibit/decrease the expression of OC-STAMP.



6. RANKL as an Inducer of OC-STAMP to Promote Osteoclast Differentiation

The receptor activator of osteoclast fusion RANKL (TNFSF11/osteoprotegerin ligand OPGL) has been identified as a type II transmembrane protein expressed on the cell surface of bone marrow stromal cells, pro-B cells and T cells (Anderson *et al.*, 1997). However, osteoblast cells are considered the main cell type that expresses RANKL to assist osteoclastogenesis (Nakashima *et al.*, 2011). RANKL interacts with the osteoclast and osteoclast precursor surfaces exclusively via RANK and then activates many signalling pathways (Takagi *et al.*, 2017a; Xiong *et al.*, 2011). RANKL is a critical factor for the regulation of osteoclastogenesis and protection of mature osteoclasts (Fuller *et al.*, 1998). Additionally, it is considered an essential bone remodelling regulator (Charles and Aliprantis, 2014).

The cell–cell interactions between osteoclast precursor cells and both osteoblasts and bone marrow stromal cells in bone induces osteoclast differentiation in vitro (Chambers *et al.*, 1993; Takayanagi, 2007). RANKL, which was identified as a membrane-bound ligand, also induced differentiation of the osteoclast in its soluble form (Miyamoto, 2013). The soluble form of RANKL, and M-CSF, a crucial cytokine for osteoclastogenesis, have been used to generate osteoclasts. M-CSF can stimulate the conversion of progenitor cells into osteoclast precursors, which can ultimately be transformed into mature osteoclasts. (Willkomm and Bloch, 2015). Furthermore, RANKL can substitute for bone marrow stromal factors in collaboration with CSF-1 to facilitate the differentiation of osteoclasts from myeloid precursors (Dougall *et al.*, 1999b).

OC-STAMP can facilitate osteoclast differentiation due to its role in osteoclast fusion. Several experiments confirmed that RANKL induces the expression of OC-STAMP protein in osteoclasts. Microarrays and real-time RT-PCR experiments have shown that in RANKL-induced osteoclast differentiation, the mRNA of OC-STAMP increases from relatively low levels to extremely high levels (Yang *et al.*, 2008). This result was confirmed using mouse OC-STAMP northern blots. Recent research has confirmed that sulforaphane (a sulphur-containing compound, SFN) strongly suppressed osteoclast differentiation (Takagi *et al.*, 2017a, b). Several proteins, including OC-STAMP, were down-regulated after SFN treatment. In addition, OC-STAMP mRNA-expression was inhibited by SFN during osteoclast differentiation (Takagi *et al.*, 2017a). OC-STAMP is thus regarded as an essential gene product for the regulation of the differentiation of osteoclasts.

7. Regulation of OC-STAMP by Factors in the RANKL Signalling Pathway

7.1. OC-STAMP Expression Requires Nuclear Factor of Activated T cells (NFATc1) in Osteoclasts:

NFATc1, also known as NFAT2 or NFATc, is a central regulatory factor of osteoclast differentiation and the downstream gene of RANKL (Song *et al.*, 2009). RANKL induces NFATc1 via the TNF receptor associated factor 6 and c-Fos signalling pathways. However, despite the inducing effect of RANKL, NFATc1 embryonic stem cell deficiency leads to failure of osteoclast differentiation, indicating that NFATc1 functions as a central modulator of osteoclastogenesis (Takayanagi *et al.*, 2002).

NFATc1 induces the expression of its target genes by binding to their promoter regions. In addition, NFATc1 can bind to its own promoter during RANKL-induced osteoclast differentiation, leading to an enhanced expression level of NFATc1 (Asagiri *et al.*, 2005). Miyamoto *et al.* (2012) have demonstrated that the mouse OC-STAMP promoter, which contains two binding sites for NFATc1, is required to induce OC-STAMP expression. In the presence of the NFAT inhibitor, FK506, NFATc1 expression was suppressed in wild type osteoclasts. Correspondingly, FK506 greatly inhibited OC-STAMP expression, indicating that OC-STAMP expression might be induced directly by NFATc1. In addition, it has been reported that OC-STAMP can be upregulated by lysophosphatidic acid (LPA) via NFATc1, indicating a direct correlation between NFATc1 and OC-STAMP (Hwang *et al.*, 2014). However, several inhibitors completely prevented RANKL-induced OC-STAMP expression, whereas the NFATc1 activation inhibitor III had no impact, indicating that the regulation of OC-STAMP mRNA expression may be independent of the NFAT signalling pathway (Kim *et al.*, 2011). Furthermore, NFATc1 mRNA expression levels of wild type and OC-STAMP knockout mice have not been reported to show any differences (Witwicka *et al.*, 2015).

7.2. Role of CD9 in Regulation of OC-STAMP:

CD9 is a membrane glycoprotein and a member of the tetraspanin protein superfamily, which is involved in a range of cell processes,

including muscle–cell fusion, myotube homeostasis, formation of myelinated axons and cell–cell fusion (Ishibashi *et al.*, 2004; Kaji and Kudo, 2004; Tachibana and Hemler, 1999). In addition, CD9 was determined to play an important role during mammalian fertilisation (Kaji *et al.*, 2002). The members of the tetraspanin superfamily that facilitate cell fusion include CD9, CD63 and CD81.

RANKL stimulated the expression of CD9 in a particular membrane lipid raft of mouse cells. It has been reported that inducing CD9 expression targeted to a specific lipid raft membrane microdomain by RANKL is crucial for cell fusion during osteoclastogenesis. Furthermore, inhibition of CD9 by a neutralising antibody or RNA interference decreases osteoclast formation, whereas over-expression of CD9 promotes cell fusion events in the absence of RANKL (Ishii *et al.*, 2006).

It has been demonstrated that OC-STAMP can potentially contribute to promoting osteoclast fusion for pathogenic bone resorption in periodontitis via upregulation of CD9 that is expressed in osteoclast precursor cells. Furthermore, reduced CD9 expression induced by RANKL was observed in osteoclast precursor cells in OC-STAMP-KO mice (Ishii *et al.*, 2018).

8. Role of OC-STAMP in Macrophage Polarisation through Signal Transducer and Activator of Transcription (STAT)6 and STAT1

The STAT family has seven members that are needed for the transduction of cytokine signals, including STAT1 and STAT6.

STAT6 expression is triggered by cytokines IL-4 and IL-13, which can facilitate macrophage fusion (Miyamoto, 2013). STAT6 is also necessary for cell–cell fusion during the formation of FBGCs, and its deficiency has been shown to inhibit their formation (Moreno *et al.*, 2007). In STAT6-deficient FBGCs, the expression of OC-STAMP was strongly inhibited. The absence of STAT6 enhanced the activation of STAT1, greatly inhibited cell–cell fusion and reduced the expression of OC-STAMP in FBGCs induced by IL-4. STAT1 deficiency in macrophages, by comparison, increased cell–cell fusion in FBGCs and raised the expression of OC-STAMP. This suggests that STAT1 and STAT6 regulate the expression of OC-STAMP and are fusogenic in FBGCs (Miyamoto, 2013).

The polarisation of macrophages has a complex mechanism that is regulated by interactions between several signalling pathways including JAK/STAT, Notch, PI3K/Akt, JNK and B7-H3/STAT3 (Szanto *et al.*, 2010). Macrophages are needed to sustain homeostasis from an immunological perspective. There are two main types of macrophages: M1 and M2. M1 macrophages intensify inflammation and tissue damage, mediate resistance to pathogens and have effective microbicidal properties (Liu *et al.*, 2014). In combination with lipopolysaccharide LPS, M1 macrophage activation is caused by cytokine IFN-γ. M2 macrophages play a crucial role in the suppression of inflammation and the facilitation of tissue repair (Mills, 2015). M2 macrophages are activated by the cytokines IL-4 and IL-13.

OC-STAMP was shown to trigger macrophage polarisation via STAT6. Knockdown OC-STAMP enhanced STAT1 activation during stimulation by LPS and the cytokine IFN-γ, while OC-STAMP overexpression raised STAT6 phosphorylation in RAW 264.7 cells. STAT1 phosphorylation was increased when OC-STAMP was knocked down in RAW264.7 cells under IL-4 induction (Yuan *et al.*, 2017). Furthermore, overexpression of OC-STAMP could substantially suppress the M1 pro-inflammatory state. Therefore, knocking down OC-STAMP could increase the number of M1 macrophages. These findings suggest that OC-STAMP controls macrophage polarisation through STAT6 and STAT1.

9. OC-STAMP Structure and Localisation

Currently, information regarding the structure of OC-STAMP is still incomplete. Recent studies consider OC-STAMP to be a significant diagnostic or therapeutic target; therefore, it is worthwhile to clarify its structure and localisation to improve that development. Microarray studies of mouse primary bone marrow cells and RAW264 detected OC-STAMP for the first time. The OC-STAMP gene is located on mouse chromosome 2H3 (Yang *et al.*, 2008b). Orthologous proteins are found in all mammalian species for which sequence is accessible. A variety of transmembrane prediction programmes suggest that OC-STAMP is a multi-pass transmembrane protein. OC-STAMP is reported to cross the plasma membrane six times with three extracellular loops and four intracellular sections. The intracellular locations of both C- and N-termini were detected using antibodies linked either to 3X FLAG Peptide or green fluorescence protein (GFP) tags, and both termini were found in the cytosol (Witwicka *et al.*, 2015).

In addition to other potential subcellular locations, CELLO2GO, a web-based system for subcellular protein localisation, classified OC-STAMP as a plasma membrane protein as shown in Table 1. The fusion protein signal was also observed in the endoplasmic reticulum and Golgi apparatus. Therefore, the precise localisation of OC-STAMP requires further investigation.

Table 1: CELLO2GO predicted OC-STAMP subcellular localisation

Localisation	Score
Extracellular	0.755
Plasma membrane	3.553
Cytoplasmic	0.096
Cytoskeletal	0.006
ER	0.067
Golgi	0.023
Lysosomal	0.082
Mitochondrial	0.195
Chloroplast	0.018
Peroxisomal	0.057
Vacouole	0.008
Nuclear	0.139

It has been shown that almost all the OC-STAMP genes found in the database have a putative N-linked glycosylation site. Additionally, the glycosylated extracellular loop would be on the plasma membrane side, as predicted by the transmembrane analysis programme. Glycosylation appears to be unnecessary for fusion under experimental conditions. It was shown that replacement of asparagine (N162) in the glycosylation loop by aspartic acid (D) made no differences to OC-STAMP in terms of its ability to rescue defective fusion in the OC-STAMP-KO osteoclasts.

10. Conclusion

This review has outlined the role of OC-STAMP in osteoclast cell–cell fusion and differentiation and macrophage polarisation. Current evidence suggests that OC-STAMP is not only a master regulator of cell fusion but may also have other important roles because it is expressed in most tissues. Determining the structure, localisation and ligands of OC-STAMP will complete the landscape and offer excellent potential to treat bone diseases by the regulation of OC-STAMP-mediated osteoclastogenesis.

Biography

Mayyadah Abdullah Alkuwayti

Department of Biological Sciences, College of Science, King Faisal University, Al Ahsa, Saudi Arabia, 00966504913032, malkwaiti@kfu.edu.sa

Dr Alkuwayti is a Sheffield graduate, Saudi assistant professor, vice dean of the Veterinary College, member of the American Society for Biochemistry and Molecular Biology (ASBMB) and member of the Saudi Association for Voluntary Work (Takatuf). She achieved sixth

place in the excellence awards in Ohio University's programme for the development of new faculty members at King Faisal University (KFU). She was a winner of the College of Science faculty member excellence award. She has published ISI/Scopus-indexed articles with the largest international publishers, including Elsevier. She has participated as an advisor on a food security hackathon at KFU. She has attended several international conferences in the UK and USA. ORCID: 0000-0002-8048-7378

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