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Acetylcholine Receptors In Mesenchymal Stem Cells

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Acetylcholine Receptors in Mesenchymal Stem Cells

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Abstract:

Mesenchymal stem cells (MSCs) are well known for their regenerative potential. Despite the fact that the ability of MSCs to proliferate and differentiate has been studied extensively, there still remains much to learn about the signalling mechanisms and pathways which control proliferation and influence differentiation phenotype. In recent years, there has been growing evidence for the utility of non-neuronal cholinergic signalling systems and that acetylcholine (ACh) plays an important, ubiquitous, role in cell-to-cell communication. Indeed, cholinergic signalling is hypothesised to occur in stem cells and ACh synthesis, as well as ACh receptor (AChR) expression, has been identified in several stem cell populations; including MSCs. Furthermore, AChRs have been found to influence MSC regenerative potential. In humans, there are two major classes of AChRs, muscarinic AChRs and nicotinic AChRs, with each class possessing several subtypes or subunits. In this review, the expression and function of AChRs in different types of MSC will be summarised with the aim of highlighting how AChRs play a pivotal role in regulating MSC regenerative function.

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List of Abbreviations

Abbreviation	Meaning
ACh	Acetylcholine
AChR	Acetylcholine receptor
AD-MSCs	Adipose-derived mesenchymal stem cells
BM-MSCs	Bone marrow mesenchymal stem cells
ERK1, ERK2	Extracellular signal-regulated protein kinases
FM-MSCs	Fetal membrane mesenchymal stem cells
iPSC	induced pluripotent stem cells
mAChRs	Muscarinic acetylcholine receptors
MSCs	Mesenchymal stem cells
nAChRs	Nicotinic acetylcholine receptors
PDL-MSCs	Periodontal ligament derived mesenchymal stem cells
RD-MSCs	Reaming debris-derived mesenchymal stem cells
SGDCs	Salivary gland derived stem cells
UC-MSCs	Umbilical cord-derived mesenchymal stem cells
WJ-MSCs	Wharton's Jelly mesenchymal stem cells

Introduction

Acetylcholine (ACh) and its receptors (AChRs) regulate cholinergic signalling in neurons. However, there is now sufficient evidence to confirm that cholinergic communication occurs in almost all mammalian non-neuronal cells [1]. However, non-neuronal cells employ ACh and AChRs in a different manner to neuronal cells. Unlike the neural cells, the uptake and synthesis of ACh and its mediated effects in non-neuronal cells occurs in an ambiguous manner and has been reviewed elsewhere [1]. Nevertheless, the non-neuronal cholinergic system has been proven to be a powerful intercellular communication tool that plays a pivotal role in numerous cellular processes. ACh, via its receptors, can modulate gene expression, cell viability, cell proliferation, cell migration and cell differentiation [1–4]. Despite being known to regulate cell differentiation, the role of the non-neuronal cholinergic system in stem cells is still relatively unexplored. Several types of stem cell express components of the non-neuronal cholinergic signalling system including functional AChRs. This includes non-neural stem cells, such as embryonic stem cells [5], hematopoietic stem cells [6], skeletal muscle stem cells [7], and MSCs [8,9].

There is sufficient evidence to conclude that MSCs express a functional cholinergic system and studies suggest a role for ACh in regulating stem cell properties [8,9]. This review will take a holistic look at cholinergic signalling mechanisms in MSCs and their influence on function with the aim of demonstrating that these cells have cholinoceptive properties which play important roles in determining MSC fate.

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Acetylcholine Receptors

There are two major classes of receptors that bind ACh and transmit its signal, namely, muscarinic and nicotinic AChRs (Figure 1). Apart from ACh, both classes of receptor bind to distinct secondary ligands that aided their identification; muscarinic receptors (mAChRs) bind muscarine and nicotinic receptors (nAChRs) bind to nicotine [10]. Both classes and their constituent subtypes permit communication between non-neuronal cells and activate signal-transduction pathways allowing maintenance of cellular function and ultimately organ homeostasis [1]. Muscarinic and nicotinic receptors have been shown to be expressed and functional in non-neuronal cells [11]. Both receptor families are membrane bound. However, they are two inherently different classes of receptor, with structural differences, resulting in regulation of differential downstream effects [1] (Figure 1). The mAChRs belong to the G-protein coupled receptor (GPCRs) family and mediate the metabotropic effects of ACh [12]. The nAChRs are ligand-gated ion channel receptors that mediate the ionotropic effects of ACh [13,14]. Both families include several subtypes or subunits, which again are expressed in a ubiquitous manner across a variety of non-neuronal cells [10,11,15,16].

The near ubiquitous AChR expression across non-neuronal cell populations have made it challenging to evaluate their role. The expression of receptor classes or subtypes varies across different non-neuronal cell types and is influenced by cell state and environmental factors [1]. Both receptor classes form an auto- and paracrine loop of ACh activity in non-neuronal cells which plays and important role in cell-to-cell communication. They may coexist in individual cells, with stimulation of one class potentially having a positive or negative effect on the other [17,18]. Furthermore, the wide-ranging influence of ACh on different types of non-neuronal cells adds to the complexity of this system [11].

Muscarinic Receptors

The mAChRs consist of five distinct subtypes referred to as type 1 - 5 (M1 - M5). These receptors are members of the GPCR family [12] (Figure 1). Once stimulated, muscarinic receptors couple to distinct species of G proteins that in turn activate second messenger signalling pathways as well as activating gated ion channels [19]. Based on downstream functionality of the coupled G proteins, they are commonly divided into two groups; stimulatory (M1, M3, and M5) or inhibitory (M2 and M4) [20]. Thus, the cellular cascade of events depends on the species of G protein with which a muscarinic receptor interacts. This, arguably, is what makes these receptors relatively slower acting compared to their nicotinic receptor counterpart [12]. The resultant downstream effects of activated muscarinic receptors are immensely complex and have widespread consequences. At almost all stages of development, mAChRs mediate the effects of ACh in almost all cells; both neuronal and non-neuronal [21]. In fact, abnormalities in mAChRs signalling are a sign of a diseased state, such as in chronic obstructive pulmonary disease, overactive bladder, or neuronal diseases such as Alzheimer's disease [22]. This and the fact that they are GPCRs have led to their study from a pharmacological point of view. Indeed, there are commercial incentives to develop research into GPCRs as a whole [23]. To date, GPCRs, including mAChRs, are the most successful therapeutically targeted family of receptors [24–26].

Nicotinic Receptors

The nAChRs are composed of multi-subunit proteins that form ligand-gated ion channels within the cell membrane [22] (Figure 1). A nAChR can be a pentamer based upon 13 possible subunits which may present as either homopentameric (consisting of 5 identical subunits) or heteropentameric (consisting of combinations of different subunits) [10,27]. There are nine α-subunits (α1 - 7, -9, and -10) and four β-subunits (β1 - 4). In addition, other subunits such as delta (δ), epsilon (ε), and gamma (γ) have also been identified in humans [8,28]. The different subunit compositions of this receptor class allow for specialised properties and diverse functions, and thus mediate numerous downstream effects [10]. Multiple nAChR subunits have been identified in non-neuronal cell populations [29,30]. Generally, these receptors are rapid acting cationic receptors that mediate a temporal opening of ion channels to allow sodium, potassium or calcium passage [10]. Consequently, an intracellular increase of such ions leads to activation of a series of signal transduction pathways. This in turn, may lead to alterations in cell proliferation, cytoskeletal rearrangement, and differentiation [31].

Mesenchymal stem cells

MSCs are multipotent adult stem cells initially isolated from bone marrow [32]. MSCs have now been isolated from various other tissues, including adipose tissue [33], dental pulp [34], peripheral blood [35], salivary glands [36], skeletal muscle [37], skin [38], and placental tissue [39–41]. The International Society of Cellular Therapy set three criteria to define stem cells as MSCs: (i) ability to adhere to plastic; (ii) expression of cell surface markers (e.g., CD73, CD90, and CD105) and lack of hematopoietic markers (e.g., CD14, CD34, and CD45) and class II major histocompatibility complex molecules; (iii) ability to differentiate down mesodermal lineages [42–44]. Generally, cultured MSCs present these features, however some differences have been observed between MSCs of different origins [45]. MSCs tend to differentiate down mesodermal lineages, however, under appropriate stimuli, it has been suggested that MSCs are capable of differentiation into tissues of endodermal and neuroectodermal lineages [42]. As MSCs are self-renewing cells with immunomodulatory properties and the ability to be differentiated into several lineages [46–49] they are a vital resource for tissue engineering, regenerative medicine, and cell-based therapy research [39,46,50].

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Expression of of mAChRs in MSCs

Bone marrow MSCs (BM-MSCs)

All five mAChRs have been identified in human MSCs. (Table 1). Hoogduijn *et al*. were the first to investigate cholinergic signalling in MSCs [8]. Using polymerase chain reaction (PCR), the authors demonstrated that human bone marrow MSCs (BM-MSCs) express the M2 receptor gene (*CHRM2*). Moreover, the authors suggested that expression was dynamic given that only half of the BM-MSCs were positive for the M2 protein. Confirmation that BM-MSCs express a functional M2 receptor was demonstrated as stimulation with muscarine increased intracellular Calcium (Ca^{2+}) concentration and downregulated production of cyclic adenosine 3',5'-monophosphate (cAMP). Intracellular Ca^{2+} and cAMP were previously proven to regulate MSC proliferation and differentiation [51]. Furthermore, muscarine induced an increase in the levels of phosphorylation of the extracellular signal-regulated protein kinases 1 and 2 (ERK1 and ERK2) [8]; the ERK1/2 pathway has been linked to control of differentiation, phosphorylating the transcription factors PPARg and RUNX2, to switch of adipose differentiation and turn on osteogenesis [52–54]. These data imply that the M2 receptor activates downstream signalling pathways that govern MSC proliferation and differentiation.

Expression of M1, M2 and M3 receptor genes (*CHRM1, CHRM2* and *CHRM3*) in BM-MSCs has also been reported [55]. Upregulated expression of these receptors after treatment with erythropoietin under both normoxic and hypoxic conditions was reported in BM-MSCs and likely marked the induced neuronal like cell differentiation. Treatment of BM-MSCs with ACh led to an increase in concentration of intracellular Ca^{2+} which was hypothesised to be mediated by M1 and M3 receptors and further influenced the phospholipase C and inositol-1,4,5-triphosphate (IP3) signalling axis. Although it was hypothesised that the effects of ACh

on BM-MSCs are mediated by the M1 and M3 receptor, the influence of other mAChRs subtypes (e.g., M2) was not investigated in detail. The M2 and M4 receptors, despite being thought to modulate inhibitory signalling pathways, have been shown to stimulate phospholipase C activity [56]. In addition, ACh is a universal cholinergic agonist, and, therefore, the influence of the nAChRs could also not be excluded.

Expression of M2 and M3 receptor genes (*CHRM2* and *CHRM3*) was reported in a study exploring mAChRs expression in human BM-MSCs, induced pluripotent stem cells (iPSCs) and MSCs derived from human iPSCs (iPS-MSCs) [28]. Interestingly, the M2 gene (*CHRM2*) is expressed in native iPSCs and during the differentiation phase into iPS-MSCs, but it was not detected at the end of the differentiation period. This implied that MSCs generated from iPSCs lose M2 expression. Consistent expression of the M2 receptor gene (*CHRM2*) in BM-MSCs was however observed despite donor-dependent variability. Expression of the M3 receptor gene (*CHRM3*) also varied during the differentiation process into iPS-MSCs. M3 receptor gene (*CHRM3*) expression was detected in native iPSCs, decreased during the differentiation process, and increased again at the end stage of differentiation into iPS-MSCs. Unlike the BM-MSCs, where the M3 receptor gene (*CHRM3*) was clearly expressed. The authors suggest that the variation in the expression profile among the different cell types might contribute to different signalling capabilities which in turn may lead to their differing biological characteristics. Variation in mAChRs expression pattern between passages and upon differentiation of MSC has indeed been reported in a study investigating human BM-MSCs [57]. Real time PCR shows downregulation in expression of the M1 (*CHRM1*) and M5 (*CHRM5*) receptor genes in consecutive passages as well as during both osteogenic and adipogenic differentiation. Furthermore, the study reported treatment

with atropine, a general muscarinic antagonist, significantly upregulated expression of the M4 receptor gene (*CHRM4*) during adipogenic differentiation of BM-MSCs.

Expression of the M3 receptor was detected at both the mRNA and protein level in mouse BM-MSCs [58]. The M3 receptor was localised primarily to the endoplasmic reticulum in the investigated BM-MSCs and as such were not competent to signal. This was confirmed in agonist studies. It may be the case that during differentiation membrane translocation occurs and enables functional M3 receptor signalling. However, this hypothesis was not investigated further. Rat BM-MSCs have been shown to express M1 and M4 receptors at the protein level and the M1 receptor was found to be localised in both the cytoplasm and cell membrane [59]. Interestingly, fluorescence-activated cell sorting (FACS) analysis only showed a third of the rat BM-MSCs expressed the M1 receptor. Treatment with ACh, a universal cholinergic agonist, caused enhanced migration of rat BM-MSCs in a dose-and time-dependent manner with no effect on proliferation. The effect of ACh on rat BM-MSCs migration was hypothesised to be mediated by the M1 receptor using atropine, a general muscarinic receptor antagonist. Indeed, activation of the M1 receptor was shown to trigger the ERK1/2 and Protein Kinase C (PKC) signalling pathways with release of Ca^{2+} which in turn regulated migration [59].

Adipose-derived MSCs (AD-MSCs)

Human adipose-derived MSCs (AD-MSCs) express the M1 and M2 receptor genes (*CHRM1* and *CHRM2*) and expression of both were upregulated following cardiogenic differentiation and denoted as markers for cardiomyocytes [60]. Interestingly, native AD-MSCs to only express the M2 receptor gene (*CHRM2*), while expression of the M1 receptor gene (*CHRM1*) was only detected once AD-MSCs were differentiated in to cardiomyocytes. Another study

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demonstrated changes in the pattern of mAChR gene expression upon AD-MSCs differentiation [61]. Expression of all five mAChR genes (*CHRM1 - CHRM5*) fluctuated throughout the differentiation of AD-MSCs into cells that expressed neural proteins. Expression levels of the M1 (*CHRM1*), M3 (*CHRM3*) and M4 (*CHRM4*) receptor genes rose during the differentiation process. In contrast, expression levels of the M2 (*CHRM2*) and M5 (*CHRM5*) receptor genes declined during the differentiation process; however, expression of the M2 (*CHRM2*) receptor gene recovered towards the end of differentiation. Furthermore, AD-MSCs isolated from rats express functional M2 receptor [9]. Rat AD-MSCs have been demonstrated to express the M1 (*CHRM1*), M2 (*CHRM2*) and M3 (*CHRM3*) receptor genes and expression of the M2 receptor was confirmed at the protein level. Stimulation of AD-MSCs with arecaidine propargyl ester hydrobromide (APE), a selective M2 agonist, caused autocrine upregulation of expression of the M2 gene (*CHRM2*). In addition, activation of the M2 receptor inhibited AD-MSC proliferation, migration, and the cell cycle. However, these effects were reversed when the agonist was withdrawn. Selectivity of APE for the M2 receptor in AD-MSCs was also confirmed using methoctramine, an antagonist with preference for the M2 receptor. Additionally, activation of the M2 receptor resulted in down regulated expression of key genes involved in cell proliferation and migration (*cyclinD1*, *PCNA*, *c-jun*, *PDGFR-β*, *CXCR4* and *CXCR7*). These findings are in agreement with the hypothesised role of the M2 receptor as an inhibitory mAChRs and suggests that M2 receptor activation places AD-MSCs in a quiescent state.

Salivary gland derived stem cells (SGDCs)

Two studies isolated the M3 receptor protein from porcine salivary gland derived stem cells (SGDCs) [62,63]. Both studies reported an increase in intracellular Ca^{2+} activity upon stimulating the porcine SGDCs with carbachol and suggested that this effect is mediated via

the M3 receptor. However, carbachol is an ACh analogue that can mimic the effect of ACh on both mAChRs and nAChRs. Both studies viewed the M3 receptor as a salivary gland marker of generated salivary gland organoids and do not report expression of other AChRs, or present data for the selectivity of carbachol to the SGDC M3 receptor.

Reaming debris-derived MSCs (RD-MSCs)

MSCs extracted from reaming debris (RD-MSCs) of male and female patients with osteoporosis, and MSCs from healthy donors, have been differentiated down osteogenic, chondrogenic, and adipogenic lineages and expression of mAChR genes was shown to be differential and dynamic [64]. Indeed, only the M4 (*CHRM4*) and M5 (*CHRM5*) receptor genes were expressed in RD-MSCs isolated from male donors, while female donors expressed the M2 (*CHRM2*), M4 (*CHRM4*), and M5 (*CHRM5*) receptor genes. RD-MSCs from female donors with osteoporosis showed no differences in mAChR expression profile to RD-MSCs from healthy female donors. However, expression of the specific subtype of mAChRs showed a degree of subject specificity in both undifferentiated RD-MSCs and RD-MSCs differentiated down specific lineages. This was hypothesised to be related to a donor-specific condition. However, the observed differences in mAChRs expression by RD-MSCs pre- and post- differentiation is compelling evidence for a role of mAChRs in regulating MSC differentiation.

Fetal membrane MSCs (FM-MSCs)

In a study that investigated mAChR gene expression in MSCs isolated from human fetal membrane (FM-MSCs), the authors report variation in expression pattern of mAChR genes between passages and upon differentiation of FM-MSCs [57]. Indeed, by passage 3, FM-MSCs demonstrated upregulated expression of the M1 (*CHRM1*) receptor gene in addition to

differentiation down both osteogenic and adipogenic lineages. Expression of the M2 (*CHRM2*) receptor gene was downregulated during differentiation of FM-MSCs down an osteogenic lineage and expression of the M3 (*CHRM3*) receptor gene was maintained throughout the differentiation process. Treatment of FM-MSCs with atropine, a general muscarinic antagonist, enhanced their viability and upregulated expression of the M1 (*CHRM1*) receptor gene during osteogenic differentiation. However, atropine treatment had no effect on the ability of FM-MSCs to differentiate down adipogenic or osteogenic lineges. The authors suggest that the M1 receptor may play an important role in differentiation of FM-MSCs. However, without selective stimulation or knockout experiments, it remains unclear which mAChRs are functional in FM-MSCs as atropine is a general muscarinic antagonist that can act on all mAChRs.

Umbilical cord-derived MSCs (UC-MSCs)

Expression of the M2 (*CHRM2*), M3 (*CHRM3*), and M4 (*CHRM4*) receptor genes was detected in human MSCs derived from the umbilical cord (UC-MSCs) [65]. Stimulation of UC-MSCs with ACh induces an intracellular Ca^{2+} response. The authors indicated that the ACh-induced response is mediated by the M3 receptor via the phosphoinositide 3-kinase (PIK3) axis. The authors viewed the M3 receptor as the best candidate to investgate Ca^{2+} intracellular signaling mediated by the PIK3 axis. This is based on how mAChR, naturally, couple to G protein and mediate downstream signalling. The M1, M3, and M5 receptors couple to G proteins known to influence Ca^{2+} mobilisation, while M2 and M4 couple to G proteins that inhibit adenylate cyclase. Indeed, the authors show data demonstrating that a selective M3-antagonist abolished the induced effects of ACh on UC-MSCs [65]. Intrestingly, the athours also reported the ability of a PIK3 inhibtor to abolish the induced

effects of ACh on UC-MSCs, suggesting that the PIK3 inhibtor might function by obstructing the ACh binding site of the M3 receptor.

In summry, the above studies suggest involvement of mAChRs in activating signalling pathways that regulate MSCs function. For example, the M2 receptor has been suggested to activate the ERK1/2 pathway in BM-MSCs [8], while the M1 and M3 receptors influence the IP3 signalling axis of the same MSCs type [55]. These data imply mAChRs can activate downstream signalling pathways that govern MSC function. However, only one study in AD-MSCs has to date has provided direct evidence for a role of mAChRs (the M2 receptor) in inhibiting proliferation, migration, and the cell cycle [9].

Expression of nAChRs in MSCs

Bone marrow MSCs (BM-MSCs)

Expression of the nAChR subunits have been reported in MSCs (Table 2). In BM-MSCs, Hoogduijn *et al*., detected gene and protein expression of the α3, α5, and α7 nAChR subunits [8]. Confirmation of functional nAChR expression was determined by stimulation with nicotine which led to an increase in intracellular calcium and an increase in the levels of phosphorylation of ERK1 and ERK2. However, this was observed in only half of the BM-MSC population upon stimulation with nicotine. It was, however, suggested that the nicotine induced effects are mediated through the α7 nAChR in BM-MSCs as the study showed an increase in levels of phosphorylated ERK in C3H10T1/2 cells (functionally similar cells to MSCs), transfected with the α7 nAChR construct, after stimulation with nicotine. Although, nicotine is a general nicotinic agonist, it remains unclear if the other nAChRs the authors identified to be expressed by BM-MSCs could have contributed to these observations.

Variation in the expression profile of nAChR genes has been reported between human BM-MSCs, iPSCs, and MSCs derived from human iPSCs (iPS-MSCs) [28]. Native iPSCs do not express the α1 subunit (*CHRNA1*) gene; however, both iPS-MSCs and BM-MSCs express transcripts of this gene. The genes for the α3, α4, α5, α7, α9, and β1 subunits (*CHRNA3, CHRNA4, CHRNA5, CHRNA7, CHRNA9,* and *CHRNB1*) were strongly expressed in iPSCs and during the generation of iPS-MSCs. However, they were only weakly expressed in generated iPS-MSCs. While BM-MSCs showed donor dependent expression of α4, α5, α7, and β1 subunit genes (*CHRNA4, CHRNA5, CHRNA7,* and *CHRNB1*), the β2 and β4 subunit genes (*CHRNB2,* and *CHRNB4*) were only expressed at low levels in iPSCs and during the generation of iPS-MSCs. Differential expression profiles of nAChR genes have been reported in human MSCs (hMSCs) [66]. Gene expression of the α1, α2, α3, α4, α5, α7, α9, β2, β3 and

β4 subunits (*CHRNA2, CHRNA3, CHRNA4, CHRNA5, CHRNA7, CHRNA9, CHRNB2, CHRNB3,* and *CHRNB4*) were confirmed in hMSCs [66]. Further analysis confirmed protein expression of the α7, β2, and β4 nAChR subunits. The study also provided evidence of functional nAChRs in hMSCs via stimulation with nicotine. Indeed, treatment with 1 μM nicotine or less induced spontaneous migration of hMSC; however, higher doses (>1 μM) caused cell death. Furthermore, the study provided evidence that nicotine inhibits the growth factors C3a and bFGF induced migration in hMSCs. Moreover, the study provided data showing the nicotine-induced effects are mediated through the α7 nAChR in hMSCs. Indeed, the α7 nAChR selective antagonist α-bungarotoxin (α-BTX) was shown to abolish the inhibitory effects of nicotine. The study also provides *in vivo* data demonstrating impaired migration of transplanted hMSCs to the bone marrow and spleen in mice as a result of nicotine exposure. Indeed, in a separate study, it has been suggested that higher doses of nicotine cause apoptosis and impair proliferation, while at non-toxic concentrations it decreases the migratory potential of MSCs [67].

A functional heteropentameric α4β2 nAChR has been reported in rat BM-MSCs [68]. Indeed, stimulation with nicotine supressed the osteogenic potential of rat BM-MSCs in a concentration-dependent manner. Nicotine (>0.1 μM) had a negative effect on the expression of osteogenesis markers such as: *Runx2*, *BSP*, *Col1*, and *OCN*. Higher concentrations of nicotine (10 μM) significantly inhibited mineralisation of differentiated rat BM-MSCs. The authors indicated that supressed rat BM-MSCs osteogenesis occurs due to nicotine promoting the activity of the angiotensin-converting enzyme (ACE) and activating the bone renin angiotensin system (RAS). This was confirmed using dihydro-β-erythroidine, a selective inhibitor of the α4β2 nAChR, which partially counteracted the nicotine-induced expression of ACE and activation of the RAS system. In a separate *in vivo* study, expression of a functional

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α7 nAChR in rat BM-MSCs has been reported [69]. The study showed that nicotine impaired the ability of BM-MSCs to repair cartilage defects in rats. Indeed, nicotine supressed chondrogenic differentiation of rat BM-MSCs as evidenced by reduced safranin-O staining of newly formed cartilage tissue. Additionally, nicotine inhibited expression of chondrogenic markers such as *Col2A1* and *Sox9* in rat BM-MSCs regenerated tissue. The authors indicate that the α7 nAChR mediates nicotine's ability to downregulate *Col2A1* expression by supressing its upstream effector *Sox9*. Indeed, the study provides evidence of involvement of the α7 nAChR in mediating the effect of nicotine as methyllycaconitine, a specific α7 nAChR antagonist, inhibited nicotine-induced Ca^{2+} influx in rat BM-MSCs. Furthermore, repressed BM-MSC chondrogenesis is thought to occur via the Ca^{2+}/calcineurin/NFATc2 signalling pathway upon nicotine stimulation. The study demonstrated that nicotine decreased cytoplasmic dephosphorylated NFATc2 with concomitant nuclear translocation of NFATc2 in response to an increase in intracellular Ca^{2+}. NFATc2 is capable of binding to the Sox9 promotor, thus decreasing *Sox9* expression. These nicotine-induced effects were abolished when BM-MSCs were pre-treated with methyllycaconitine, indicating the involvement of the α7 nAChR in attenuating the Ca2+/calcineurin/NFATc2 signalling pathway.

Adipose-derived MSCs (AD-MSCs)

Human AD-MSCs express both the α and β nAChR subunits [61]. Expression of the α7 and β4 nAChR subunits (*CHRNA7*, and *CHRNB4*) were upregulated while the α3, α6, and β2 nAChR subunits (*CHRNA3, CHRNA6,* and *CHRNB2*) were significantly downregulated in neuronal differentiated AD-MSCs. Functional nAChRs were confirmed as nicotine induced an increase in intracellular Ca^{2+}, most significantly when AD-MSCs underwent neuronal differentiation. However, no data eluding to which nAChRs mediate the effect of nicotine is reported. In another study, AD-MSCs derived from rats were found to express a functional α7

nAChR [70]. Indeed, stimulation with ICH3, a selective α7 nAChR agonist, inhibits rat AD-MSCs proliferation. Further analysis confirms that the α7 nAChR inhibits AD-MSCs proliferation by promoting cell cycle arrest via downregulation of *Cyclin D1* expression. However, activation of the α7 nAChR significantly enhanced rat AD-MSCs migration via upregulation of CXCR4, a chemokine receptor that also mediates cellular migration. Both these effects could be counteracted using α-BTX, an α7 nAChR selective antagonist. Interestingly, ICH3 treatment of AD-MSCs also increased protein expression of the M2 mAChR suggesting a potential cross-interaction mechanism between m and n-AChRs.

Periodontal ligament derived MSCs (PDL-MSCs)

Human periodontal ligament derived MSCs (PDL-MSCs) have been reported to express the α7 and β4 nAChR subunit genes (*CHRNA7*, and *CHRNB4*) and nicotine stimulation amplifies their expression [71]. Nicotine stimulation had a negative impact on PDL-MSCs viability in a dose dependent manner. Higher concentrations of nicotine, > 100 μM, were associated with increased DNA fragmentation in PDL-MSCs and accumulation of cells in subG1 phase of the cell cycle, the phase associated with apoptosis. Indeed, nicotine in millimolar levels activated apoptotic pathways in PDL-MSCs and increased expression of p53, a pro-apoptotic marker, was evident after only 30 min treatment with 10 mM nicotine. This was associated with a decrease in levels of the Bcl-2 anti-apoptotic protein and an increase in the well-known apoptotic marker caspase-3. However, the nicotine-induced apoptosis was blocked when PDL-MSCs were pre-treated with α-BTX, the aforementioned α7nAChR-specific antagonist. Thus, confirming the role of the α7 nAChR in mediating the nicotine-induced effects on apoptosis in PDL-MSCs. Data from a study by Zhou *et al* confirmed that PDL-MSCs express a functional α7 nAChR and nicotine inhibited PDL-MSCs proliferation in a dose dependent manner [72]. Moreover, stimulation with nicotine dose-dependently impaired osteogenic

differentiation of PDL-MSCs. Indeed, differentiated PDL-MSCs showed significant decreases in bone mineralisation associated with decreased expression of osteogenic genes and protein markers (ALP, OCN, BSP, and RUNX2). However, the nicotine-induced impairment of differentiation was partially reversed by α-BTX, suggesting that the α7 nAChR regulates, to an extent, PDL-MSCs osteogenic differentiation. In fact, both gene and protein expression of the α7 nAChR in osteo-differentiated PDL-MSCs is increased when nicotine is present. This suggests the involvement of the α7 nAChR and cholinergic signalling in the process of osteogenesis. In fact, several *in vivo* and *in vitro* studies in chick and mouse have reported involvement of ACh dependent pathways regulating skeletogenesis and bone development [73–75]. In which it was suggested that α7 nAChR mediated the nicotine inhibitory effects on cartilage and bone formation [74]. All which are supportive of a role for cholinergic regulation in bone development. In addition, nicotine-stimulated nAChRs can initiate relevant downstream signalling pathways. Indeed, it was shown that the α7 nAChR mediate the downstream effects of nicotine through the wnt/β-catenin pathway in PDL-MSCs [72]. Nicotine stimulation of PDL-MSCs lead to a decrease in protein expression of wnt-related factors, DKK-1 and GSK-3β, and an increase in the expression of active-β-catenin protein. The latter has been previously shown in a separate study to suppress PDL-MSCs osteogenic differentiation [76]. However, in the presence of α-BTX all these effects were reversed, again providing further evidence of a functional α7 nAChR modulating the wnt/β-catenin pathway in PDL-MSCs [72].

Wharton's Jelly MSCs (WJ-MSCs)

Human Wharton's Jelly MSCs (WJ-MSCs), derived from the mucous connective tissue between the amniotic epithelium and the umbilical vessels found in the umbilical cord, express genes encoding the α3, α5, α7, β2, and β4 nAChR subunits (*CHRNA3, CHRNA5,*

CHRNA7, CHRNB2, and *CHRNB4*) [77]. WJ-MSCs, in response to nicotine treatment, demonstrate significantly decreased proliferation but no change in viability, apoptosis or necrosis. The chondrogenic differentiation capacity of WJ-MSCs was impaired, to an extent, by nicotine. Indeed, while nicotine treatment did not affect the collagen output of differentiated WJ-MSCs, it did impair the quality of the collagenous matrix formed, as determined by the proteoglycan content. This was asserted to be due to the downregulated expression of chondrogenic markers including *Sox9, Col2a1* and *aggrecan*. The authors suggested that the α7 nAChR mediated the nicotine-induced effects in WJ-MSCs as it induced Ca^{2+} influx into the cells. In a separate study, human WJ-MSCs were confirmed to express a functional α7 nAChR as well as the α3 and α9 nAChR subunit genes (*CHRNA3,* and *CHRNA9*) [78]. Furthermore, injection of human WJ-MSCs into α7 nAChR deficient mice demonstrated improved episodic memory and suggest increased regenerative potential of WJ-MSCs to improve cognitive functions via the α7 nAChR.

Reaming debris-derived MSCs (RD-MSCs)

Expression profile data available for nAChRs provides interesting insight into variation dependent upon sex and health of RD-MSC donors [64]. For example, the α5, α7, and α9 nAChR subunit genes (*CHRNA5, CHRNA7,* and *CHRNA9*) were expressed in RD-MSCs isolated from all donor groups (male and female, healthy and diseased). In contrast, the α2, α6, and α10 nAChR subunits (*CHRNA2, CHRNA6,* and *CHRNA10*) were only expressed in RD-MSCs isolated from diseased female donors and the α3 nAChR subunit gene (*CHRNA3*) was only expressed in RD-MSCs isolated from diseased male donors. The study also reported variations in expression of certain nAChR subunits between different differentiated lineages. However, the α7 subunit gene (*CHRNA7*) was expressed in RD-MSCs differentiated down all lineages (osteogenic, chondrogenic, and adipogenic lineages). Furthermore, the α3 subunit

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gene (*CHRNA3*) was expressed in adipocytes generated from female RD-MSC donors; but not by adipocytes generated from male RD-MSC donors. Therefore, it can be concluded that the expression profile of the nAChR subunits is dynamic in RD-MSCs.

In summary, the aforementioned studies provide evidence that MSCs express functional nAChRs that have been shown to mediate the impact of nicotine. These in turn activate signalling pathways such as the ERK1/2 [8], Ca^{2+}/calcineurin/NFATc2 [69], and wnt/β-catenin pathway that are involved in MSC function [72]. However, there is limited direct evidence for a role of a specific nAChR in translating the effect of nicotine. One example of a demonstrated direct effect is in the case of the α7 nAChR which appears to mediate nicotine inhibitory effects on hMSCs migration [66]. Additionally, impaired chondrogenic differentiation of BM-MSCs in response to nicotine has been demonstrated to be mediated through α7 nAChR [69]. Interestingly, the α7 nAChR was shown to inhibit AD-MSCs proliferation however enhances migration [70]. In PDL-MSCs, the α7 nAChR mediates nicotine-induced apoptosis [71], and to an extent impaired these cells' ability to undergo osteogenic differentiation [72]. However, in BM-MSCs the α4β2 nAChR was shown to supress cells osteogenic potential in response to nicotine [68].

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Conclusion

The studies presented in this review show the widespread expression of AChRs in MSCs and demonstrates the involvement of these receptors in MSCs function. It appears that AChR expression in MSCs is dynamic, dependent on the type of MSCs, and can be individually based on donor or differentiation lineage. However, expression of all mAChR subtypes have been identified in most of MSCs studied as well as both the α and β nAChR subunits. Furthermore, consistant expression of a particular AChR subtype across different types of MSCs has been observed. For example, the M3 mAChR and α7 nAChR are expressed in multiple types of MSCs. This may suggest central roles of these receptors in regulating MSC function.

Downstream signalling of AChRs in MSCs:

Some of the presented studies examined the downstream effects of AChR activation on MSCs regenerative potential. In stem cells therapy, the regenerative output is determined by the ability of the cells to migrate, proliferate, and differentiate. The presented findings show involvement of major pathways involved in regulating these functions. In the MAPK/ERK pathway, phosphorylation of ERKs are known to regulate proliferation and differentiation of stem cells [79]. As described herein, both muscarinic and nicotinic AChRs were shown to be involved in triggering this signalling pathway cascade in MSCs. Indeed, as AChR activation facilitates downstream signalling pathways that influence cell homeostasis, they have the capability to influence the regenerative potential of MSCs. However, it is the presence of a subtype functional AChR that determines specific downstream signalling cascade activation and determine cell fate. In the case of mAChRs, downstream signalling is mostly dependent on the coupled G protein (Figure 2). Indeed, the stimulatory group of mAChRs (i.e., M1, M3,

and M5) coupled to Gq proteins mainly influence Ca^{2+} influx in MSCs. While the inhibitory group of mAChRs (M2 and M4) coupled to Gi proteins mainly influence cAMP production. Furthermore, both groups of mAChRs share a common downstream pathway for ERK1/2 activation. Dependent on the mAChRs subtype, this can result in promoting MSCs function, e.g., M1 regulating BM-MSCs migration [59], or inhibiting MSCs growth, e.g., M2 inhibiting AD-MSC proliferation [9].

In the case of nAChRs, these receptors are mainly gated ion channels, for example the most characterised in MSCs is the α7 nAChRs which modulates intracellular $Ca2^{+}$ concentration. Most of the reviewed studies examined downstream effects of nAChRs activation through nicotine. It may have been the focus of these studies to determine the impact of nicotine on the overall potential of treated MSCs. Indeed, nicotine had an overall negative impact on MSCs regenerative potential. The majority of the studies identified the α7 nAChR in mediating nicotine's effects on MSCs. The consequent changes in the intracellular Ca^{2+} concentrations can initiate several signalling cascades (Figure 3). This can involve MAPK effectors, via the PI3K pathway or in conjugation with other pathways, such as the calcineurin/NFATc2 or wnt/β-catenin pathways. In most of the studies, this results in inhibition of MSC growth or even in initiation of apoptosis. While other studies show inhibition or suppression of MSCs potential to differentiate as seen with chondrogenic differentiation of BM-MSCs [69] and osteogenic differentiation of PDL-MSCs [72].

AChRs as a potential target to regulate MSCs function

The evidence suggests that AChRs may present as a promising therapeutic target to control the regenerative potential of MSCs. Indeed, the reviewed studies presented data for subtype-selective AChRs agonist and antagonists in manipulating stem cell function. For example, the

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selective M2 agonist APE placed AD-MSCs in a quiescent state without affecting the viability of the cells [9]. This may be favourable during transplant and for directing *in vivo* regeneration. Migration, an important function of stem cell therapy during *in vivo* regeneration, can be controlled via AChRs. indeed, the M2 selective agonist APE supressed AD-MSCs migration via the M2 mAChRs [9]. Another promising option is to block undesirable AChR function through selective and non-selective agonists. For instance, atropine the general mAChRs antagonist was able to enhance FM-MSCs viability by blocking the M1 receptor [57]. It is not unexpected that mAChRs have this central role in MSCs, these receptors were shown to be influential in many body systems e.g., nervous, cardiovascular, and muscular [80]. As mAChRs are GPCRs, they belong to the most successful therapeutic targeted family of proteins that continue to be the most prominent aim of biomedical research [24–26].

Non excitable cells are now known to express a plethora of ion channels, and these are also interesting targets for pharmacological intervention. Here, MSCs were shown to express several subunits of nAChRs among which there are functional ones that are susceptible to pharmacological stimulation. Indeed, the selective α7 nAChR agonist ICH3, was shown to enhance MSCs migration [70]. Likewise, α-BTX, the α7 nAChR selective antagonist had the ability to block the detrimental effects of nicotine in several MSCs [67,71,72]. These data display an interesting potential for targeting ion channels that influence MSC function. It is also worth mentioning that other classical excitable ion channels are being investigated in control of MSC phenotype. Worthy of mention are the Piezo ion channels, which regulate osteogenesis of MSCs by regulating the expression of *BMP2* [81] and migration via adenosine triphosphate (ATP) release [82]. Likewise, of interest are the Transient Receptor

Potential (TRP) ion channels which can influence MSCs differentiation [83], cell cycles [84], and survival [81].

Acetylcholine signalling, mediated by muscarinic and nicotinic AChRs, is indeed involved in regulating MSC function. Targeting both muscarinic and nicotinic AChRs with pharmacological agents may therefore reveal novel mechanisms to tune MSC function and thus their regenerative output. Indeed, there is a plethora of AChRs agonists and antagonists licensed for the treatment of a variety of diseases [25,80,85,86] and these have yet to be explored with regards to their effects on the regenerative potential of MSCs and the possibility of their re-purposing into the regenerative medicine arena. Furthermore, there is more to learn about the metabotropic downstream signalling of mAChRs in MSCs and indeed even if they do play a naturally occurring role in MSC homeostasis or differentiation. It is therefore imperative that more studies are undertaken to investigate the precise functions of AChRs in MSCs.

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Authorship contribution statement

SA: Conceptualization (equal); writing - original draft (lead).
MCB: Funding acquisition (lead); review and editing (equal).
GR: Writing – review & editing (equal); Supervision (equal).
MJD: review and editing (equal); Writing - original draft (supporting).
WM: Conceptualization (supporting); writing - review and editing (equal); Supervision (equal).
CJN: Conceptualization (supporting); writing - review and editing (equal); Supervision (equal)

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Conflicts of interest/Competing interests

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7 Figure 1 Schematic presentation of ACh receptors. Left: Muscarinic acetylcholine receptors
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9 (mAChR) are G-protein coupled receptors. Based on downstream functionality of the coupled
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11 g proteins they are commonly divided into two groups, stimulatory in nature (M1, M3, and
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14 M5) or inhibitory (M2 and M4). Right: Nicotinic acetylcholine receptors (nAChR) are
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16 pentamers from 16 possible subunits. They may present as either homopentamers (consisting
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18 of 5 identical subunits) or heteropentamers (consisting of combinations of different subunits).
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23 Figure 2 Metabotropic signaling of muscarinic receptors. Upon stimulation with
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25 acetylcholine, or a subunit specific agonist, M1, M3 and M5 receptors activate Phospholipase
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27 C (PLC) resulting in downstream protein kinase C (PKC) activation and an increase in IP3
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29 and Ca^{2+} levels. PKC can also activate the MAPK cascade and ERK1/2. M2 and M4 subtypes
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32 inhibit the activity of the adenylyl cyclase, leading to a decrease in intracellular cAMP. In
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34 addition, mAChRs can activate ion channels. Common pathways for all mAChRs are the
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36 activation of ERK1/2 via a Src/PI3K pathway. The M2 subtype may also modulate Akt
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39 singling by means of upstream PI3K activation, influencing transcription factors regulating
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41 proliferation.
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44 Figure 3 Diagram depicting the α7 nAChR signaling pathway. Upon stimulation with
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47 acetylcholine, or a subunit specific agonist, nAChRs increase cytosolic ca^{2+} concentration,
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49 initiating several signaling cascades. The Ca^{2+} influx with activation of calcineurin can
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51 induce NFATc dephosphorylation and translocation back to the nucleus. This recruits
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54 transcription factors that govern cell differentiation. Similarly, the α7nAChR, via the
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56 wnt/AKT pathway, can translocate β-catenin into the nucleus and subsequently activate
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58 expression of target genes that modulate differentiation. Common signaling pathways
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activated by nAChRs include the MAPK via PI3K pathway. This can occur in a Fyn dependent manner or by means of upstream phosphorylation of JAK2. Additionally, Inhibition of JAK2 activates caspase 3, leading also to activation of the MAPK downstream signaling pathway. Later effectors such as NF-$_{K}$P, ERK, and Bcl-2 signal nuclear transcription factors (e.g., PARP, Bax/BaK, and p53) were found to play a role in the downstream signaling of α7 nAChR activation. Depending on the downstream signaling pathways activated these pathways can impinge on transcription factors that control synthesis and repair of DNA or promote apoptosis; consequently, influencing cell proliferation, migration, and survival.

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Table 1 Summary of muscarinic acetylcholine receptors (mAChRs) in MSCs. N/A: not assessed.

Muscarinic subtype	Species	mRNA expression	Protein expression	Functional expression
M1	Human	BM-MSCs [55,57] FM-MSCs [57] AD-MSCs [61]	N/A	BM-MSCs [55]
	Rat	AD-MSCs [9] BM-MSCs [59]	BM-MSCs [59]	BM-MSCs [59]
M2	Human	BM-MSCs [8,28,55] FM-MSCs [57] AD-MSCs [60,61] RD-MSCs [64] UC-MSCs [65]	BM-MSCs [8]	BM-MSCs [8]
	Rat	AD-MSCs [9]	AD-MSCs [9]	AD-MSCs [9]
M3	Human	BM-MSCs [28,55,57] iPS-MSCs [28] FM-MSCs [57] AD-MSCs [61] UC-MSCs [65]	N/A	BM-MSCs [55] UC-MSCs [65]
	Rat	AD-MSCs [9]	N/A	N/A
	Mouse	BM-MSCs [58]	BM-MSCs [58]	N/A
	Porcine	N/A	SGDCs [62,63]	SGDCs [62,63]
M4	Human	BM-MSCs [57] RD-MSCs [64]	N/A	N/A
	Rat	BM-MSCs [59]	N/A	N/A
M5	Human	BM-MSCs [57] AD-MSCs [61] RD-MSCs [64]	N/A	N/A

Table 2 Summary of nicotinic acetylcholine receptors (nAChRs) in MSCs. N/A: not assessed.

Nicotinic subunit	**Species**	**mRNA expression**	**Protein expression**	**Functional expression**
α1	Human	BM-MSCs [28] iPS-MSCs [28] MSCs [66]	N/A	N/A
α2	Human	MSCs [66] RD-MSCs [64]	N/A	N/A
α3	Human	BM-MSCs [8] AD-MSCs [61] RD-MSCs [64] MSCs [66] WJ-MSCs [77]	BM-MSCs [8] WJ-MSCs [78]	N/A
α4	Human	BM-MSCs [61] MSCs [66]	N/A	N/A
α5	Human	BM-MSCs [8,28] iPS-MSCs [28] RD-MSCs [64] MSCs [66] WJ-MSCs [77]	BM-MSCs [8]	N/A
α6	Human	AD-MSCs [61] RD-MSCs [64]	N/A	N/A
α7	Human	BM-MSCs [8,28] iPS-MSCs [28] RD-MSCs [64] AD-MSCs [61] MSCs [66] PDL-MSCs [71,72] WJ-MSCs [77]	BM-MSCs [8] MSCs [66] PDL-MSCs [72] WJ-MSCs [77,78]	BM-MSCs [8] MSCs [66] PDL-MSCs [71,72] WJ-MSCs [77,78]
	Rat	BM-MSCs [69] AD-MSCs [70]	BM-MSCs [69] AD-MSCs [70]	BM-MSCs [69] AD-MSCs [70]

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α9	Human	iPS-MSCs [28] MSCs [66]	WJ-MSCs [78]	N/A
α10	Human	RD-MSCs [64]	N/A	N/A
β1	Human	BM-MSCs [28]	N/A	N/A
β2	Human	AD-MSCs [61] MSCs [66] WJ-MSCs [77]	MSCs [66]	N/A
β3	Human	MSCs [66]	N/A	N/A
β4	Human	AD-MSCs [61] MSCs [66] PDL-MSCs [71] WJ-MSCs [77]	MSCs [66]	N/A
α4β2	Human	BM-MSCs [68]	N/A	BM-MSCs [68]

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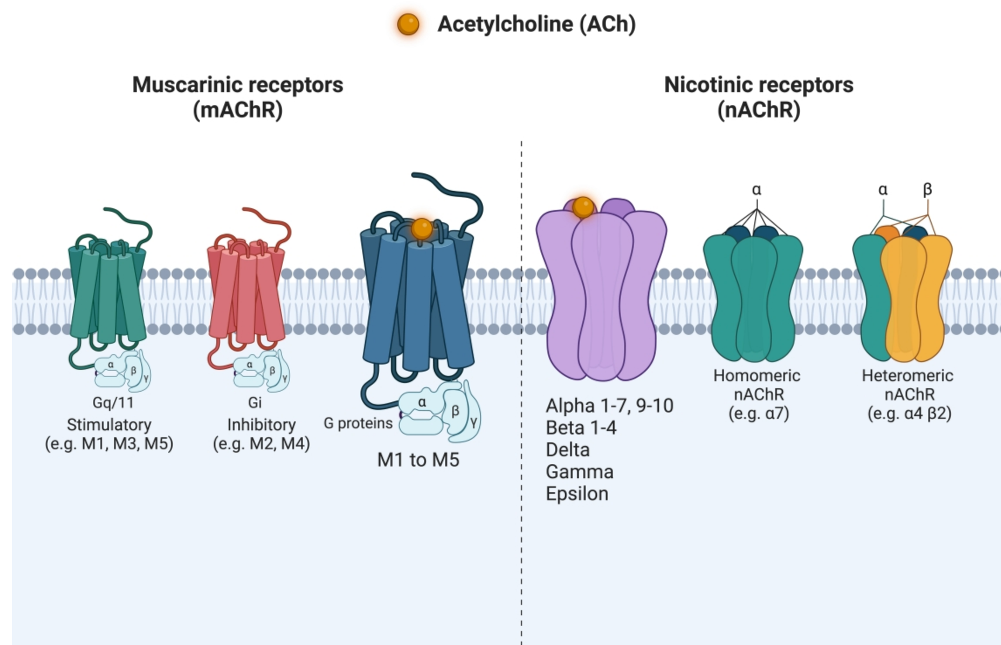

Schematic presentation of ACh receptors. Left: Muscarinic acetylcholine receptors (mAChR) are G-protein coupled receptors. Based on downstream functionality of the coupled g proteins they are commonly divided into two groups, stimulatory in nature (M1, M3, and M5) or inhibitory (M2 and M4). Right: Nicotinic acetylcholine receptors (nAChR) are pentamers from 16 possible subunits. They may present as either homopentamers (consisting of 5 identical subunits) or heteropentamers (consisting of combinations of different subunits).

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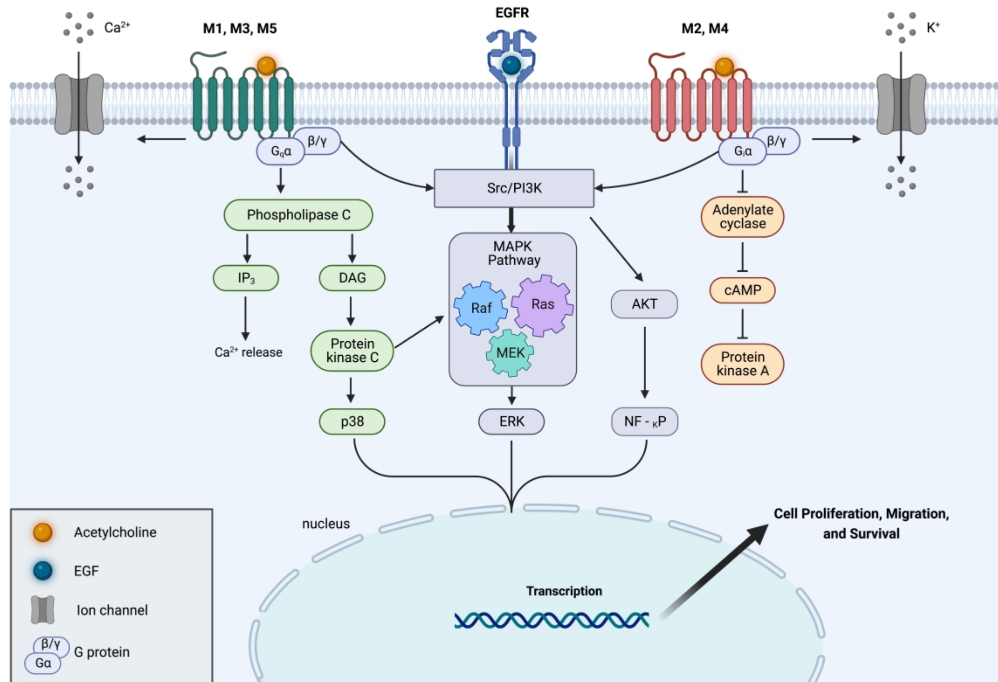

31 Metabotropic signaling of muscarinic receptors. Upon stimulation with acetylcholine, or a subunit specific
32 agonist, M1, M3 and M5 receptors activate Phospholipase C (PLC) resulting in downstream protein kinase C
33 (PKC) activation and an increase in IP3 and Ca2+ levels. PKC can also activate the MAPK cascade and
34 ERK1/2. M2 and M4 subtypes inhibit the activity of the adenylyl cyclase, leading to a decrease in
35 intracellular cAMP. In addition, mAChRs can activate ion channels. Common pathways for all mAChRs are
36 the activation of ERK1/2 via a Src/PI3K pathway. The M2 subtype may also modulate Akt singling by means
of upstream PI3K activation, influencing transcription factors regulating proliferation.

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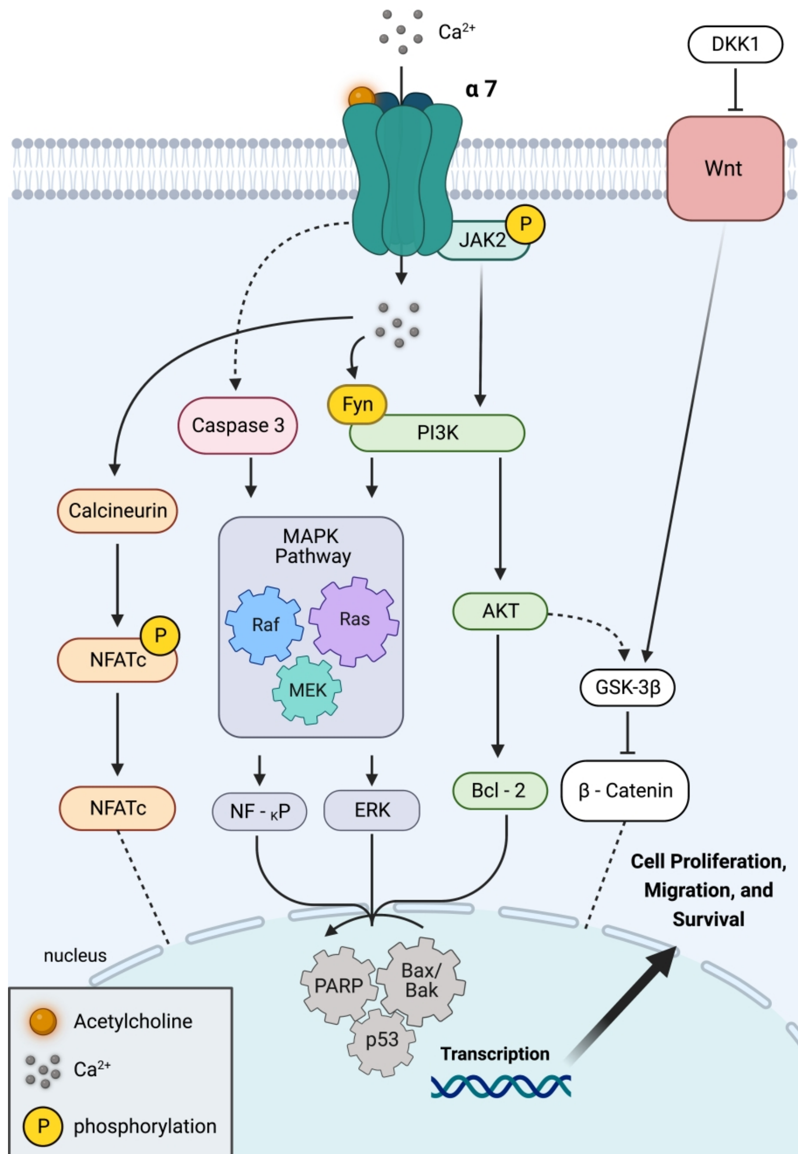

Diagram depicting the α7 nAChR signaling pathway. Upon stimulation with acetylcholine, or a subunit specific agonist, nAChRs increase cytosolic ca2+ concentration, initiating several signaling cascades. The Ca2+ influx with activation of calcineurin can induce NFATc dephosphorylation and translocation back to the nucleus. This recruits transcription factors that govern cell differentiation. Similarly, the α7nAChR, via the wnt/AKT pathway, can translocate β-catenin into the nucleus and subsequently activate expression of target genes that modulate differentiation. Common signaling pathways activated by nAChRs include the MAPK via PI3K pathway. This can occur in a Fyn dependent manner or by means of upstream phosphorylation of JAK2. Additionally, Inhibition of JAK2 activates caspase 3, leading also to activation of the MAPK downstream signaling pathway. Later effectors such as NF-KP, ERK, and Bcl-2 signal nuclear transcription factors (e.g., PARP, Bax/BaK, and p53) were found to play a role in the downstream signaling of α7 nAChR activation. Depending on the downstream signaling pathways activated these pathways can impinge on transcription factors that control synthesis and repair of DNA or promote apoptosis; consequently, influencing cell proliferation, migration, and survival.

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Table 1 Summary of muscarinic acetylcholine receptors (mAChRs) in MSCs. N/A: not assessed.

Muscarinic subtype	Species	mRNA expression	Protein expression	Functional expression
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	Rat	AD-MSCs [9] BM-MSCs [59]	BM-MSCs [59]	BM-MSCs [59]
M2	Human	BM-MSCs [8,28,55] FM-MSCs [57] AD-MSCs [60,61] RD-MSCs [64] UC-MSCs [65]	BM-MSCs [8]	BM-MSCs [8]
	Rat	AD-MSCs [9]	AD-MSCs [9]	AD-MSCs [9]
M3	Human	BM-MSCs [28,55,57] iPS-MSCs [28] FM-MSCs [57] AD-MSCs [61] UC-MSCs [65]	N/A	BM-MSCs [55] UC-MSCs [65]
	Rat	AD-MSCs [9]	N/A	N/A
	Mouse	BM-MSCs [58]	BM-MSCs [58]	N/A
	Porcine	N/A	SGDCs [62,63]	SGDCs [62,63]
M4	Human	BM-MSCs [57] RD-MSCs [64]	N/A	N/A
	Rat	BM-MSCs [59]	N/A	N/A
M5	Human	BM-MSCs [57] AD-MSCs [61] RD-MSCs [64]	N/A	N/A

Table 1 Summary of nicotinic acetylcholine receptors (nAChRs) in MSCs. N/A: not assessed.

Nicotinic subunit	Species	mRNA expression	Protein expression	Functional expression
α1	Human	BM-MSCs [28] iPS-MSCs [28] MSCs [66]	N/A	N/A
α2	Human	MSCs [66] RD-MSCs [64]	N/A	N/A
α3	Human	BM-MSCs [8] AD-MSCs [61] RD-MSCs [64] MSCs [66] WJ-MSCs [77]	BM-MSCs [8] WJ-MSCs [78]	N/A
α4	Human	BM-MSCs [61] MSCs [66]	N/A	N/A
α5	Human	BM-MSCs [8,28] iPS-MSCs [28] RD-MSCs [64] MSCs [66] WJ-MSCs [77]	BM-MSCs [8]	N/A
α6	Human	AD-MSCs [61] RD-MSCs [64]	N/A	N/A
α7	Human	BM-MSCs [8,28] iPS-MSCs [28] RD-MSCs [64] AD-MSCs [61] MSCs [66] PDL-MSCs [71,72] WJ-MSCs [77]	BM-MSCs [8] MSCs [66] PDL-MSCs [72] WJ-MSCs [77,78]	BM-MSCs [8] MSCs [66] PDL-MSCs [71,72] WJ-MSCs [77,78]
	Rat	BM-MSCs [69] AD-MSCs [70]	BM-MSCs [69] AD-MSCs [70]	BM-MSCs [69] AD-MSCs [70]
α9	Human	iPS-MSCs [28] MSCs [66]	WJ-MSCs [78]	N/A

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α10	Human	RD-MSCs [64]	N/A	N/A
β1	Human	BM-MSCs [28]	N/A	N/A
β2	Human	AD-MSCs [61] MSCs [66] WJ-MSCs [77]	MSCs [66]	N/A
β3	Human	MSCs [66]	N/A	N/A
β4	Human	AD-MSCs [61] MSCs [66] PDL-MSCs [71] WJ-MSCs [77]	MSCs [66]	N/A
α4β2	Human	BM-MSCs [68]	N/A	BM-MSCs [68]