Short Communication

Molecular characteristics of purinergic receptors and its mediated signaling, a novel target for stem cell-based therapy: A brief summary

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Abstract

Molecular identity of nucleotide factor(s) that serve as trigger for the activation of cell-cycle progression in adult stem cells or human mesenchymal stem cells (hMSCs) or progenitor cells is not well understood. Recent studies highlight the importance of extracellular nucleotide-triphosphates (eNTPs) like ATP as key regulators of various functions executed by the adult stem cells. It has long been speculated that discrete release of ATP by the cells in response to shear stress plays a major role in the initiation of various physiological processes such as proliferation, repair and regeneration, migration and homing. eNTPs acts via purinoceptors (P2Y) and multiple isoforms of P2Y receptor are expressed in all the cells, the molecular mechanisms of P2Y-signaling still remain unexplored. On certain stimuli, hMSCs release ATP constitutively and ATP activates variety of P2X/P2Y receptors in MSCs inducing robust Ca^{2+} responses from different tissues/species and calcium mediated functional responses in hMSCs were considered as predominant one. The mechanism of store-operated Ca^{2+} entry through which the intracellular calcium concentration is restored plays a major role in that. Yet the cognate intrinsic mechanisms of these purinergic receptors remain contentious. Our interest is to understand the basic mechanisms under which the stem cells perform their functions by strengthening the signaling cascades and genetic transcription effected by activation of purinoceptors.

Keywords: ATP receptors, calcium signalling, human mesenchymal stem cells

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Introduction

Extracellular ATP related nucleotides behave as trophic, immunomodulatory molecules through its purinergic receptors: P1 receptors for nucleosides and P2 receptors for nucleotides. Purinergic ligands are well recognized mediators of autocrine/paracrine signaling involved in proliferation, migration, immunomodulation and regeneration.[1] An upcoming interest in translational research is on cell based therapieswith human mesenchymal stem cells (hMSCs), a cell-population with self-renewal and multipotency that was derived initially from the bone marrow, later from the adipose tissues, Wharton's jelly/placenta and dental pulp.[2-8] The isolated cells are recognized as hMSCs only after confirming their morphology and functional characteristics by series of tests that specifically proves their adherence ability, positive expression for specific surface markers(CD), the clonogenic assays and differentiating abilities maintaining their lineage properties.[5,9,10] This review briefly discuss the recent advances in molecular characterization of purinergic receptors, their established novel physiological

processes and how they act as ideal candidates to mediate the crosstalk with resident stem cell niches, promoting cell growth and survival, differentiation, immunomodulation, migration and repair.

Extracellular Purines - A Heterodox Concept

From an evolutionary point of view, the most ancient recognized molecules with autocrine/paracrine signaling are the extracellular nucleotides (eNTPs). They are released during stress/hypoxia and comprises of both extracellular purines (ATP, ADP, β-NAD, ADPR and cADPR) and extracellular pyrimidines (UTP and UDP) in the extracellular mileu.[1] Most recent research is being highly focused on the role of purine nucleotide ATP (enzyme cofactor). In 1929, ATP was first isolated by Karl Lohmann and documented as signaling molecule by Drury andSzent.[11-13] Only decades later, ATP was accepted as potent extracellular messengers producing effects on energy metabolism, signal transduction and cellular communication.[14-16] The release of eNTPs is regulated by leakage through ATP-binding cassette transporters, connexin hemichannels, or voltage-dependent anion channels or by receptor mediated exocytosis.[17] It was proven that eNTPs bind a distinct cell surface receptors, the Purinergic receptor family.[13]

Purinergic receptors

Purinergic receptors, the oldest receptors, were initially reported in gut smooth muscles in 1970s.[18-20] Purinoceptors have been proved to have role in cell proliferation, differentiation and migration in endothelial cells, tumour cells, embryonic or adult stem cells.[21-23] Based on the order of activation of agonists, agonists efficacy and desensitization characteristics to its antagonists the purinoceptors were classified into P1 receptors (adenosine receptors) with its natural ligand adenosine and P2 receptors(nucleotide receptors) activated by other nucleotides(ATP, UTP).[24] The seven trans-membrane domain P1 receptors comprises of A1, A2A, A2B and A3, mediates its effects via phospholipase C, adenylyl cyclaseand mitogen-activated protein kinase pathway.[25,26]

Ectoenzymes: Currently, an accumulating evidence suggests that a family of ectonucleotidases (E-NTPases or ectonucleoside triphosphate diphosphohydrolases) called ectoenzymes on the plasma membrane, play a role in the nucleotide metabolism.[27-29] Once released, ATP mediates signaling via purinoceptors or degraded to nucleosides (AMP, adenosine) by the sequential action of four ectoenzymes: ectonucleotide pyrophosphatase, ectonucleotide triphosphate diphosphohydrolase/CD39 (E-NTPDase), alkaline phosphatase, and ecto-5'-nucleotidase/CD73.[30-33]

P2 receptors exist as two distinct families: The metabotropic P2Y and ionotropic P2X receptorsare widely distributed on both excitable and nonexcitable mammalian cells. P2Xare cation-selective, ligand-gated ion channels (LGIC) on the plasma membrane activated by ATP and P2Y are G-protein coupled receptors activated by nucleotides, di-phosphates or triphosphates, purines and pyrimidines.[34,35] However, several studies states that seven out of eight members of the P2X receptor family (P2X1–7) expressed in splice variants and approximately six out of 11 types of P2Y receptors (P2Y1, P2Y2, P2Y4, P2Y6, P2Y11, and P2Y12) expressed were documented to have functional responses.[13] P2 and P1 receptors mediate varied signaling events, where the ligand and receptor affinity of P2 and P1 differs from nanomolar to micromolar levels.[36,37]

- P2=affinity range from nanomolar (e.g., P2Y2) to micromolar (e.g., P2X7) levels;
- P1=affinity range from low nanomolar (e.g., A1,A2A,A3) to high nanomolar (e.g., A2B) levels.

P2X and P2Y receptors: Purinergic signaling is also modulated by cross-talk between P1 and P2 receptors.[38] Studies have well documented the role of ATP via P2R in hMSC differentiation.[39-41]

- P2Y receptors in the adipogenic/osteogenic differentiation of hMSCs.[42]
- P2X receptors on proliferation(P2X1/5) and differentiation of hMSCs (P2X5 and P2X7).[23]

Of the seven different subunits (P2X1–7)P2X1-5 are homomeric receptors and P2X2/3 and P2X1/5 receptors were heterodimer, with the exception of P2X6 and P2X7.[43-46]Functional responses P2X receptors are mediated by increasing intracellular calcium ion concentration, yet P2X7 was proven to mediate via MAP-kinase, heat shock proteins, and phosphatidylinositol 4-kinase.[47-50] The P2Y metabotropic, heptahelical G-protein coupled receptor (GPCR) family has eight subtypes in human tissues-P2Y1, P2Y2, P2Y4, P2Y6, P2Y11, P2Y12, P2Y13 and P2Y14 and are activated by nucleotides like

ADP,ATP, UDP and UTP.[51] They couple to Gq and activate phospholipase C-β (P2Y-1,2,4,6,11), or to Gi(P2Y-12,14)that inhibit adenylyl cyclase whereas P2Y11 are coupled to both Gq/Gsand increase intracellular Ca2 and cAMP levels.[52]

Purinoceptors in MSCs

MSCs are constituitively activated by ATP upon stimuli and the presence of purinergic receptors and ectoenzymes on their plasma membrane.[53-55] Evans and coworkers were the first who evidenced the presence of P1 receptor in stem cell surface.[56] Both invitro and invivo studies have put forth the role of P1 receptors in the process of adipogenic differentiation (A1,A2A), osteogenic differentiation (A2B) and cytokine activation in Bone marrow MSCs by gene knockout studies as well as pharmacological deletion/blockade of P1 receptors.[57-64]

Advances in the molecular biology are used to identify novel ligands for P2 receptors and its relation to hMSC has gained attention only recently. Studies states that the spontaneous release of ATP from MSCs occurs via pannexins hemichannels (gap junction) on the plasma membrane and its direct stimulation of P2Y1 receptor was associated with increase in intracellular Ca^{2+}levels.[55] This study hypothesized that modulation of the proliferation rate of MSCs at early passages cultures could be achieved by the concurrent activation of P2X and P2Y receptors by ATP analogs/agonists.[23] A gene knockout study in rat MSCs suggested that the activation of P2Y2 receptor induced intracellular Ca2+ responses that directly correlated with cell cycle progression.[65] Currently, an in-vivo study by Ferrari and collaborators observed an upregulation of pro-inflammatory cytokines and cell migration related genes in ATP pretreated MSCs, enhanced the migratory and homing ability of hMSCs, this concept was considered supportive in therapies for bone marrow engraftment.[66]

Plausible purinergic cascade in hMSCs[22,39,41,43,55]

- eNTPs increases the intracellular cytosolic Ca^{2+} levels ($[Ca^{2+}]i$) in hMSCs via enhancing Ca^{2+} influx through plasma-membrane-calcium-channels(PMCA) and hemichannels (pannexins) as well as Ca^{2+}release from intracellular stores of mitochondria and endoplasmic reticulum (ER).
- eNTPs binds to G-protein-coupled-P2Y receptors, can elevate the $[Ca^{2+}]i$ by stimulating phospholipase-C to generate inositol-1,4,5-triphosphate(IP3), which in-turn activates the IP3 receptor on endoplasmic reticulum (ER) and induces Ca^{2+} release from ER.
- Thus the reduction Ca^{2+} level inside ER activates the Stim1 channels on ER-membrane which aggregates and coactively binds to ORAI-1 channels in plasma membrane causing increased Ca^{2+} influx-intracellularly. This mechanism called Ca^{2+} release-activated Ca^{2+} channels (CRAC) enables reconstituition of cytosolic Ca^{2+} levels. STIM1 and ORAI-1 are the essential components of CRAC channels that mediate store-operated^{2+} Ca^{2+}entry (SOCE) in cells.
- Increases in the concentration of either calcium induce cAMP pathway that will further trigger the activation of kinasesa and cAMP responsive element binding protein, a nuclear protein that modulates the transcription of genes for chemotaxis, cellular motility, proliferation, immunomodulation and repair.

Methods of study [58,66-69]

Expression of purinoceptors, ion channel and transduction pathways in hMSCs can be studied by Western blot method and qPCR. Immunocytochemistry will help in studying the underlying cytokines. Recording of intracellular calcium concentration and functioning of calcium ion channels can be studied by single cell imaging using fura-2 based radiometry. This method will clearly delineate the mechanism of ER-Ca^{+}stores depletion by using specific and nonspecific Calcium-ATPase blockers cyclopiazonic acid (CPA) or thapsigargin(TG) to Orai1/Stim1channels. Animal models by method of knockout-mice (gene specific for the targeted receptor is deficient or deleted) has been the best proven method to assess the functionale of purinoceptors in the absence of selective antagonists or antisense probes.

P2 receptors and ectoenzymes are dynamic cellular entities. Studies have proved that P2Y receptors and ecto-59-nucleotidase, ecto-apyrase and E-NTPaseundergo stage-specific transient expression during cellular movement.[67] Studies are becoming increasingly evident on the role of altered E-NTPase

activities as novel drug targets. Apart from ligand binding assays, functional fluorescent imaging (FLIPR) in cell lines transfected animal models has been suggested the advanced screening approach to identify newer ligands to human P2 receptors.[68,69]

Conclusion

Researchers have their current interests on identifying the crosstalk between biochemical signals produced by MSCs in tissue environments. However, some have revealed unexpected findings about eNTPs and their derivatives in modulating the physiology of stem cells. From what has been discovered so far, a brief insight about the purinergic receptors has been summarized. We hope that this will help researchers to precisely define these molecular signals focusing their importance for future hMSC-based therapies.

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Conflicts of Interest: Nil

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