channels are inhibited by cytosolic concentrations of $Ca^{2+}$ in a CaMKII-dependent manner. Supported by NSERC & the Jeanne Mance Foundation, Hotel Dieu Hospital.

**1358-Pos Board B202**

**Characterization of Transient Receptor Potential Melastatin 7 in Bone Marrow Stem Cells**

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Changes in the intracellular concentrations of $Ca^{2+}$ and $Mg^{2+}$ play a significant role in cell growth and differentiation. Mesenchymal stem cells (MSCs) from bone marrow are a potential source for tissue repair due to their ability to differentiate into specialized cells, including bone, fat and muscle. However, the molecular signals controlling the differentiation process remains largely unknown. In this study, we examined whether MSCs express Transient Receptor Potential Melastatin 7 (TRPM7), a member of the TRP family of ion channels and a key pathway for $Ca^{2+}$ and $Mg^{2+}$ entry into cells. By RT-PCR, we identified TRPM7 transcripts with the expected molecular size of 198bp, but not TRPM6 (317bp), a close family member with similar function. Electrophysiological recordings revealed that depletion of intracellular $Mg^{2+}$ or $Mg^{2+}$-ATP activated TRPM7, suggesting that the channel is functionally active. Furthermore, treatment of MSCs with 2-aminoethoxydiphenyl borate (2-APB 1pM-100μM), a TRPM7 blocker inhibited TRPM7 currents in a dose-dependent manner. Our findings suggest that TRPM7 may represent an important pathway for controlling stem cell growth and differentiation by regulating the amount of $Ca^{2+}$ and $Mg^{2+}$ entering cells.

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**The Type IV Mucolipidosis-Associated Protein TRPML1 is an Endolysosomal Iron Release Channel**

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TRPML1 (mucolipin-1/MCOLN1) is predicted to be an intracellular late endosomal and lysosomal ion channel protein belonging to the mucolipin subfamily of Transient Receptor Potential (TRP) proteins. Mutations in the human *TRPML1* gene cause mucolipidosis type IV disease (ML4). ML4 patients exhibit motor impairment, mental retardation, retinal degeneration, and iron-deficiency anemia. Since aberrant iron metabolism may cause neural and retinal degeneration, it may be a primary cause of ML4 phenotypes. In most mammalian cells, release of iron from endosomes and lysosomes following iron uptake via endocytosis of $Fe^{3+}$-bound transferrin receptors, or following lysosomal degradation of ferritin-Fe complexes and autophagic ingestion of iron-containing macromolecules, is the major source of cellular iron. The Divalent Metal Transporter protein (DMT1) is the only endosomal $Fe^{2+}$ transporter currently known and is highly expressed in erythroid precursors, but genetic studies suggest the existence of a DMT1-independent endosomal/lysosomal $Fe^{2+}$ transport protein. Here, by measuring radiolabeled iron uptake, monitoring the levels of cytosolic and intra-lysosomal iron and directly patch-clamping the late endosomal/lysosomal membrane, we show that TRPML1 functions as a $Fe^{2+}$ permeable channel in late endosomes and lysosomes. ML4 mutations are shown to impair TRPML1's ability to permeate $Fe^{2+}$ at varying degrees, which correlate well with the disease severity. A comparison of TRPML1$^{-/-}$ ML4 and control skin fibroblasts showed a reduction of cytosolic $Fe^{2+}$ levels, an increase of intra-lysosomal $Fe^{2+}$ levels, and an accumulation of lipofuscin-like molecules in TRPML1$^{-/-}$ cells. We propose that TRPML1 mediates a mechanism by which $Fe^{2+}$ is released from late endosomes/lysosomes. Our results suggest that impaired iron transport may contribute to both hematological and degenerative symptoms of ML4 patients.

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**Activation Mutations of the TRPML1 Channel Revealed by Proline Scanning Mutagenesis**

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The mucolipin TRP (TRPML) proteins are a family of intracellular channels primarily localized in the late endosome and lysosome. Mutations in the human *TRPML1* gene cause mucolipidosis type IV disease, a devastating pediatric neurodegenerative disease. In wild-type TRPML1-expressing HEK293 cells, no significant channel activity can be detected at the plasma membrane, but a proline substitution (TRPML1$^{V432P}$) results in a large whole-cell current that allows characterization of TRPML1 as a $Ca^{2+}$ and $Fe^{2+}/Mn^{2+}$ dually permeable channel. As TRPML1-mediated current can be recorded in late endolysosom using our recently developed lysosome patch-clamp technique, it remains unknown whether large TRPML1$^{V432P}$ mediated current has resulted from increased surface expression (''trafficking'' effect), increased constitutive channel activity (''gating'' effect), or both. In the current study, we systematically but individually performed the proline substitutions on 20 amino acid residues around the *432* spot, a S4-S5 linker region. These proline-substitutions were studied by whole-cell and lysosome lumenal-side-out recordings in TRPML1-expressing HEK293 cells. Several proline substitutions were identified to display gain-of-function (GOF) constitutive activity at both the plasma membrane and endolysosomal membranes, and their localizations were not restricted to late endosomes and lysosomes, while wild-type TRPML1 and non-GOF substitutions were localized exclusively in these compartments. All of the proline-substituted GOF TRPML1 channels displayed inwardly rectifying currents that were carried by $Ca^{2+}$ or $Fe^{2+}/Mn^{2+}$, but not protons. As lysosomal exocytosis is known to be $Ca^{2+}$-dependent, constitutive $Ca^{2+}$ permeability of proline substitutions may have resulted in stimulus-independent intralysosomal $Ca^{2+}$ release, hence the surface expression and whole-cell current of TRPML1. We conclude that the TRPML1 channel is an inwardly rectifying proton-impermeable cation-permeable channel, which may be gated through unknown cellular mechanisms through a conformational change in the cytoplasmic face of the TM5.

**1361-Pos Board B205**

**Dynamic Properties of the TRPML3 Pore and their Modification by the Varitint-Waddler Phenotype**

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TRPML3 is a $Ca^{2+}$ channel expressed in intracellular vesicular compartments and is regulated by $H^+$ that interact with the large intravesicular loop between transmembrane domains 1 and 2. The A419P mutation in TRPML3 causes the varitint-waddler phenotype as a result of gain-of-function (GOF). The mechanism by which the A419P mutation leads to GOF is unknown. Here, we show that the TRPML3 pore is dynamic and expands when conducting $Ca^{2+}$ to change its permeability and selectivity from a strong to a weak field strength site. Pore expansion appears to be regulated by trapping $Ca^{2+}$, probably within the pore. Expansion of the pore can be reversed only by conducting $Na^+$ through the pore. The A419P mutation, which locks the channel in an open state results in permanently expanded pore. Notably, the TRPML3(H283A) mutation that eliminates regulation of TRPML3 by $H^+$ and locks the channel in an open state shows the same pore properties as wild-type TRPML3. On the other hand, the pore mutation E449A also locks the channel in an open state and permanently expanded pore. Interestingly, the TRPML3 large intravesicular loop interacts with the pore domain composed of transmembranes 5 and 6. Although this interaction is enhanced by the A419P and E449A mutations, it is not affected by the loop mutation H283A, suggesting that pore expansion together with enhanced loop-pore communication is responsible for the GOF. These findings provide a molecular mechanism for GOF by the TRPML3(A419P) mutation to account for the varitint-waddler disease phenotype.

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**Regulation by Calcium of the TRP Channel Polycystin-2 (TRPP2)**

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• Polycystin-2 (PC2, TRPP2) is a member of the TRP (transient receptor potential) superfamily of cation channels. Like other members of this superfamily, PC2 permeates $Ca^{2+}$, which is involved in both signal transduction, and $Ca^{2+}$ entry. Previously, we showed that PC2 is normally active at intracellularly high $Ca^{2+}$ concentrations (10-15 μM). Little is known, however, about the role intracellular $Ca^{2+}$ plays in PC2 channel function. Here, we explored the role of physiological concentrations of intracellular $Ca^{2+}$ in PC2-mediated channel function in reconstituted apical membranes from term human syncytiotrophoblast (hST). Addition of either EGTA (1 mM) or BAPTA (2 mM) to reach low intracellular $Ca^{2+}$ (<5 nM) at the cytoplasmic side, elicited a complete PC2 channel inhibition. A dose response elicited by addition of increasing cytoplasmic $Ca^{2+}$ showed that $Ca^{2+}$ activated PC2 with an apparent half activating concentration of 4.78 nM and a Hill coefficient of ~5. Conversely, extracellular $Ca^{2+}$ concentrations, between 0.5 mM and 5 mM, had a stimulatory effect on PC2 channel activity while higher external concentrations