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CHARACTERIZATION OF PULMONARY STEM CELLS AS MODEL FOR VIRUS INFECTIONS

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We have recently identified a rare subpopulation of lung cells (0.004%) with the characteristics of pulmonary stem /progenitor cells, which appear to interact with their surrounding mesenchymal stroma cells to maintain their phenotypes as stem cells (PNAS, 103: 9530-9535; 2006). In this study, we report an optimized serum-free culture system that supports the growth of Oct-4$^+$ epithelial colonies in primary pulmonary cultures. Epithelial-like colonies appeared in these serum-free conditions after 10-14 days of culture with low concentration of EGF in the medium. The presence of Oct-4 mRNA was confirmed by quantitative RT-PCR performed with the colony cells plucked from the cultures to evaluate the level of Oct-4 expression in the pulmonary colonies. It was found that the Oct-4 expression in these pulmonary colony cells was high, about 51, 52 and 88% of those expressed in ES cell lines 46c, R1 and J1 respectively,. In addition to Oct-4, these cells also express other stem cell markers such as Nanog, SSEA-1, and Sca-1, but not c-Kit, CD34 and p63. Furthermore, these morphologically unique colony cells can be cultured in vitro for months and maintain characteristics of stem cells through interaction with surrounding stroma cells. On the other hand, once free from surrounding cells, they undergo differentiation to become more mature pneumocytes sequentially. The cells plunked from individual colonies can also be subcultured onto irradiated stroma cells and continued to express Oct-4 for days. Therefore, the surrounding cells, which expressed α-smooth muscle actin, CD44 and CD90, and can partially been induced to become adipocytes, appear as some form of mesenchymal cells. In addition, we have demonstrated the presence of Oct-4$^+$, long term BrdU label retaining cells at the bronchoalveolar junction, providing a link between the Oct-4$^+$ cells in vivo and in vitro and enforcing their identity as putative lung stem cells. Thus, these primitive pulmonary cells represent a population of slow cycling, Oct-4$^+$ expressing cells, scattering at bronchoalveolar junctions of lung tissues. Finally, we recently found that these pulmonary colony cells were infected not only by SARS-CoV, but also by Hantavirus, and influenza viruses (e.g. H1N1, H2N2 and H5N1), exhibiting the specific susceptibility of the colony cells toward virus infection. The results of such virus infections may account for the deterioration of lung tissues and the apparent loss of capacity for lung repair upon some respiratory viral infections. Our finding demonstrate that this culture system could also be a good model for drug selection for respiratory infection and would be useful for studying interactions with surrounding stroma, which may play important roles(s) in lung remodeling.

Key Words: Pulmonary stem cells, viral infections, Oct-4 expression

S 3-1

THALAMIC mGluR1-PLC β4 CASCADE IN INFLAMMATORY PAIN

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Nociceptive input is integrated and modulated at multiple peripheral, spinal and supraspinal synaptic junctions. Considerable attention over the past decay has been focus on the molecular mechanisms of pain processing at the dorsal ganglion and spinal level. It is, however, poorly understood the molecular mechanisms of the pain processing at supraspinal level. We revealed that metabotropic glutamate receptor type 1 (mGluR1) and phospholipase C (PLC) β4 cascade is crucial for the formalin-induced inflammatory pain by regulating the response of neurons in the mouse ventroposterolateral thalamic nucleus (VPL). Interestingly, mGluR1 is located on the postsynaptic site of corticothalamic synapses, but not on the lemniscal (spinothalamic) synapses. In this talk, we discuss how the cortico-thalamic input is involved in coding the inflammatory pain information by mGluR1-PLCb4 cascade.