Ikuo Morita

1 .Topic in Research Achievements in the Year 2006

1. Cell Differentiation induced by connexins

We showed that Connexin43 (Cx43), a major gap junction protein, is crucial for cell differentiation in vitro. Cx43 short hairpin RNA (shRNA) dramatically inhibited cell differentiation. Conversely, Cx43 overexpression facilitated the differentiation of cells. Furthermore, Cx43, especially its C-terminal, regulated cell differentiation via the accumulation of cyclic adenosine monophosphate (cAMP).

2. A novel method of DDS using gap junction

We developed a new route for direct cytosolic delivery of hydrophilic drugs through gap junction channels (GJs) formed between liposomes containing connexins and intact cells in culture. Cx43-containing liposomes were prepared by using cell-free transcription/translation systems with plasmids encoding Cx43 in the presence of liposome. The expressed membrane protein, Cx43, was directly reconstituted to the liposome membrane upon in vitro synthesis, leading to pure membrane protein-containing liposomes. Transfer of the hydrophilic dye calcein between Cx43-expressing liposomes and cultured cells was observed when GJs were established. The transfer through heterotypic GJ between Cx43 and Cx32 was less functional than their homotypic pair. The system is useful as a tool in cell biology research and as a new drug carrier as cellular cytosolic delivery.

3. A novel angioplasty using printing technique

Mononuclear cells (MNC) were isolated from peripheral blood, and differentiated into two kinds of EPCs: early EPCs and late EPCs. These EPCs and Mature endothelial cells HUVECs were applied to our novel methods for measuring tube forming activity. Cells were cultured onto the fully patterned plates, and to form vascular tube, the plate was contacted with Matrigel. Late EPCs as well as HUVECs exhibited tube formation with similar morphology, whereas early EPCs failed to form any tubes. It is interesting that when early or late EPCs were cultured with the tube formed by HUVECs, HUVECs could be substituted by late EPCs, but not by early EPCs. However, sprouting of HUVEC was observed by the addition of early EPCs. In conclusion, early EPCs promote migration and proliferation of endothelial cells, whereas late EPCs constitute blood vessels. It suggests that circulating EPCs have different roles in neovascularization.

4. Mechanism for altered production of cytokines under hypoxia

Hypoxia is a microenvironmental pathophysiologic factor commonly associated with tissue inflammation. We found that hypoxic stress actively regulates cytokines' expression not only by activation of specific genes, but also by selective repression. In particular, we found an increase in IL-8, but decrease in MCP-1 mRNA and protein expression. Inconsistent with mRNA expression, in reporter gene assay both of the promoters were activated by hypoxia. This suggests the critical role of in vivo chromatin modification in the regulation of MCP-1 gene under hypoxia. NF-□B was responsible for changes in both MCP-1 and IL-8 mRNA expressions by hypoxia. We found that in hypoxic cultures HDAC was the corepressor that converted NF-□B activating signal into repressing towards MCP-1 gene expression. HDAC1 or HDAC3 to perform their inhibitory function. Cotreatment with IL-1 and hypoxia facilitated the association of p65/RelA and CBP to IL-8 promoter and increased histone H4 acetylation. Hypoxia repressed IL-1-stimulated MCP-1 expression by recruiting p65/RelA, HDAC3 and corepressor N-CoR, and deacetylation of histones H3 and H4. Therefore, hypoxic signal driven by NF-□B can inhibit or induce gene expression depending on selective recruitment of corepressors or coactivators.

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