Determining RNA Interference in Mouse Embryonic Stem Cells and Their Differentiated Cells Using a Novel dsRNA-Based Antiviral Assay

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Embryonic stem cells (ESCs) are a promising cell source for regenerative medicine because of their unlimited proliferation capacity and ability to differentiate into any cell type. However, recent studies have shown that ESCs do not have a normal interferon (IFN)-based-antiviral mechanism. Since the IFN response is a vital antiviral pathway in most mammalian somatic cells, the lack of this mechanism raises concerns for the medical application of ESCs and raises questions on what mechanisms protect ESCs against viral infection. One possible mechanism that ESCs may utilize is RNA interference (RNAi), an antiviral pathway used by invertebrate cells but is uncertain in mammalian cells. This project aims to develop a double-stranded RNA (dsRNA)-based virus-free antiviral assay to determine if RNAi is functional in ESCs. The principle of this method is that synthetic long dsRNA can act as a viral RNA analog and evoke antiviral response when introduced into cells. In this assay, Green Fluorescent Protein (GFP) was expressed in ESCs as an artificial viral protein. ESCs were transfected with synthetic dsRNA corresponding to the sequence of GFP (dsGFP) or luciferase (dsLuc, which has a different sequence and was used as a control). Both dsRNA induced non-specific antiviral response through activation of dsRNA-activated-kinase. However, only dsGFP caused the sequence-specific reduction of GFP expression typically seen with RNAi activity. Interestingly, dsGFP-mediated RNAi activity was not detected in ESC-differentiated-fibroblasts. These results provide evidence for the existence of RNAi in ESCs and supports the hypothesis that mammalian ESCs and differentiated cells use different antiviral mechanisms.

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