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Gene Silencing in mammalian embryonic stem cells using RNA interference technology

Tamal Bhaumik

RNA interference (RNAi) is a post-transcriptional silencing process that works by causing homologous, short RNA species to degrade messenger RNA (mRNA) transcripts. Since its discovery in 1998 by Andrew Fire and Craig Mello, RNAi has been the focus of considerable research, with two main drivers. The first reason is that RNAi is a long-lived, evolutionarily conserved gene-control mechanism that is thought to exist in many if not all, eukaryotic model systems. It has been demonstrated to be important in a variety of functions, from embryonic gene expression regulation to viral immunology. The second reason why RNAi has piqued the interest of scientists and biomedical researchers is because of its practical uses, both in the lab and in therapeutics. Researchers primarily focused on investigating whether long, double-stranded RNA (dsRNA) could trigger an RNAi response in Caenorhabditis elegans and plants. These investigations found that dsRNA could mute homologous mRNA transcripts, resulting in a reduction in gene-specific expression. According to previous studies, long dsRNA causes a non-specific immunological response in many types of mammalian cells, which is mediated by a dsRNA-dependent protein kinase (PKR). As a result, rather than sequence-specific mRNA degradation, as found in C. elegans, the PKR-directed interferon pathway can cause a global translation and apoptosis shutdown. In 2001, gene-specific RNAi silencing in mammalian tissue culture cells was achieved by introducing shorter dsRNA species (<21 kilobases) into the cells. Short interfering RNAs (siRNAs) can inactivate genes specifically by blocking mRNAs, causing their degradation and reducing the interferon response. This finding made it possible to employ RNAi-based methods to manipulate gene expression in mammalian systems on a massive scale. It is worth mentioning, however, that dsRNA does not activate the interferon system in all mammalian cell types. Long dsRNA has been used to silence specific genes in mouse oocytes/zygotes, embryonic stem (ES) cells and embryonal carcinoma (EC) cells. Owing to this, the development of cell and gene therapies combining RNAi and ES cells is currently achievable. Concerns about the limited number and purity of cells available for cell replacement therapies may be eased by ES cell research. Thus, understanding ES cell differentiation is crucial. RNAi can be utilised to find genes involved in pluripotency and lineage commitment. Genes involved in the formation of specific lineages can also be downregulated to enrich pure cell cultures and prevent unwanted derivation. The tumorigenicity of ES-derived cell lines can be decreased by eliminating genes linked with cell proliferation. RNAi may also be used to manipulate the immune system and reduce the rejection of ES cell transplants. Finally, using RNAi in ES cells may assist in modelling diseases in vitro and finding new therapeutic targets. A combination of RNAi and ES cells could lead to many intriguing discoveries in the next few years.

Keywords: Messenger RNA, Double-stranded RNA, Gene expression, Embryonic stem cell, RNA interference

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