RECOMBINANT VACCINIA VIRUS INTERFERON INHIBITOR B18R: COMPONENT OF EPIGENETIC REPROGRAMMING COCTAILS

A. Baltabekova[1]
aliya_sko@mail.ru

National Center for Biotechnology (Astana, Kazakhstan)

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Introduction: The B18R protein of *Vaccinia virus* binds to type I interferons and inhibits activation of interferon-mediated signal transduction. For a number of reasons related to the use of virus-based vectors for epigenetic reprogramming, the B18R has become a ubiquitous component of cocktails of factors which are added to primary cell cultures to achieve the epigenetic reprogramming. Little information has been published on the obtaining of the recombinant B18R. Market prices for the B18R are very high, e.g. USD579 for 50 ug (ThermoFisher Cat# 34-8185-81).

We developed a procedure for bacterial expression, refolding and purification to produce the biologically active B18R. The method allows producing of milligram quantities of the recombinant B18R which is biologically active as it allows replication of the RNA-vectors in the primary human fibroblasts (HFF).

Methods: Gene for the B18R with 6His-tag was produced *de novo*. Synthetic gene was cloned into expression plasmid pET28c. The B18R was extracted from inclusion bodies using sodium lauroyl sarcosinate (SLS) as a solubilizing agent. Protein was subject to oxidative refolding in the presence of $CuSO_4$. SLS was removed using anion-exchange chromatography on Q-sepharose. Polishing purification was done using immobilized metal affinity chromatography. Thus produced B18R was added to HFF cultures which were transfected with autonomously replicating RNA (RNA-replicon) which produces GFP during intracellular replication.

Results: Autonomously replicating RNAs are incapable of replication in primary cells (such as HFF) because of the development of strong interferon-mediated response. Transfection of HFFs with RNA-replicon does not lead to GFP expression if the culture is maintained in plain medium w/o interferon inhibitors. Upon addition of 500 ng/ml of the B18R into culture media, >90% of cells preserve GFP-fluorescence during serial passages. Again, if the B18R is omitted, these cells quickly loss fluorescence indicating the elimination of the RNA-replicon.

Conclusion: The bacterially expressed protein B18R requires refolding to become biologically active. The B18R helps stably maintain autonomously replicating RNAs in primary cell cultures.