β-Actin Gene Silencing via siRNA and Its Effects on Protein Profiles

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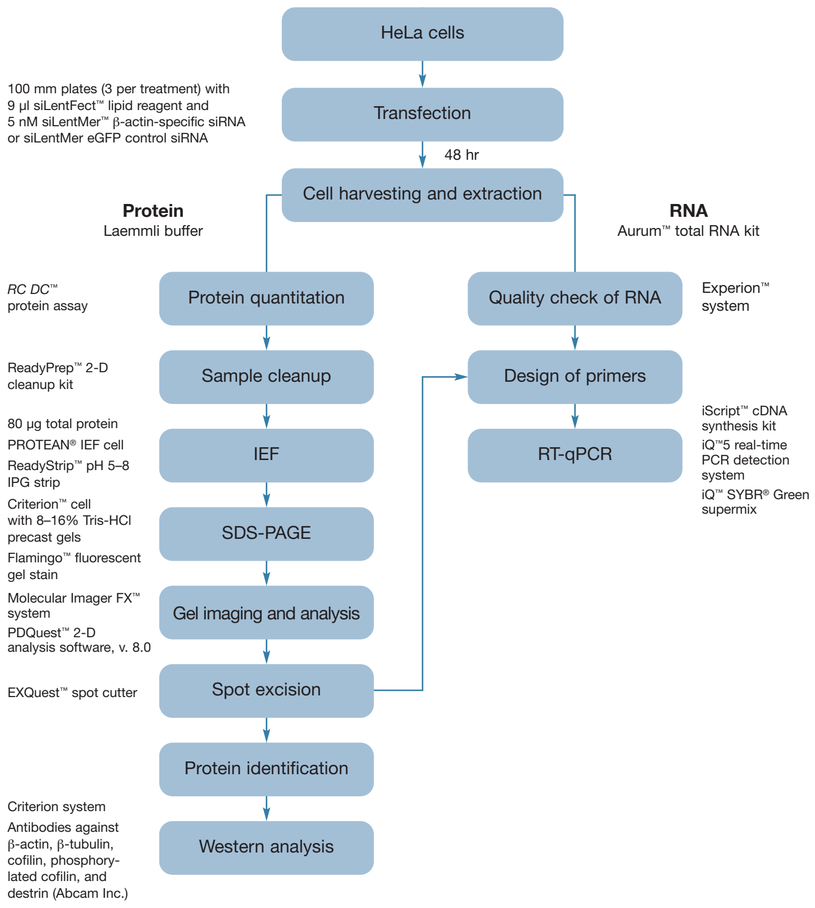
Introduction

RNA interference (RNAi) is a powerful tool used to modulate gene expression and determine gene function. Delivery of small interfering RNA (siRNA) into cells can result in degradation of a targeted messenger RNA (mRNA) and reduction of its protein product. Resulting changes in mRNA levels can be effectively monitored by RT-qPCR, while two-dimensional gel electrophoresis (2-DGE) allows the monitoring of changes in the expression levels of proteins associated with the function of that gene in a cellular pathway.

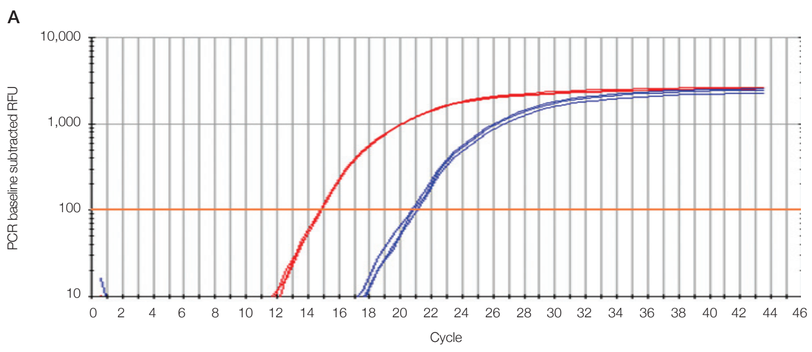
Actin filaments are major cytoskeletal structures that play important roles in many cell physiological behaviors, such as migration, proliferation, and differentiation. The proper function of actin is dependent on the highly dynamic assembly and disassembly of its filaments. Many proteins interact with actin to regulate the cytoplasm through crosslinking, bundling, capping, or severing of actin filaments (Weeds 1982).

In this study, we examine changes in the protein profiles of HeLa cells after siRNA-mediated knockdown of β-actin. Any changes in protein expression resulting from β-actin knockdown may be directly or indirectly associated with actin filament function.

Experimental Workflow



Results



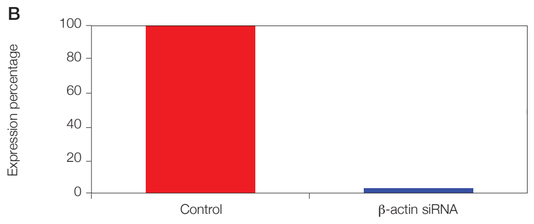
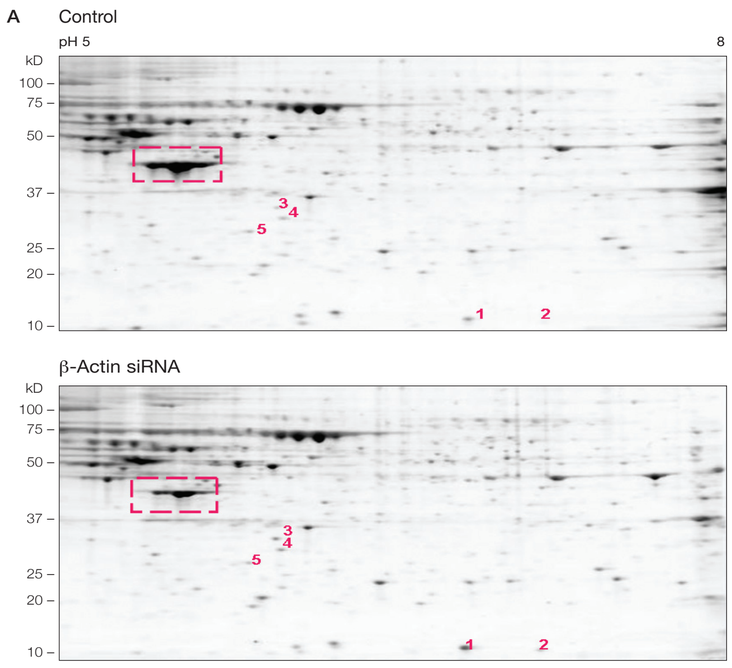


Fig. 1. RT-qPCR analysis of β-actin expression. A, RT-qPCR traces; B, relative expression levels of the β-actin message. RT-qPCR analyses showed that the expression of β-actin mRNA (—) was reduced after transfection with the β-actin-specific siRNA compared to the eGFP control (—).



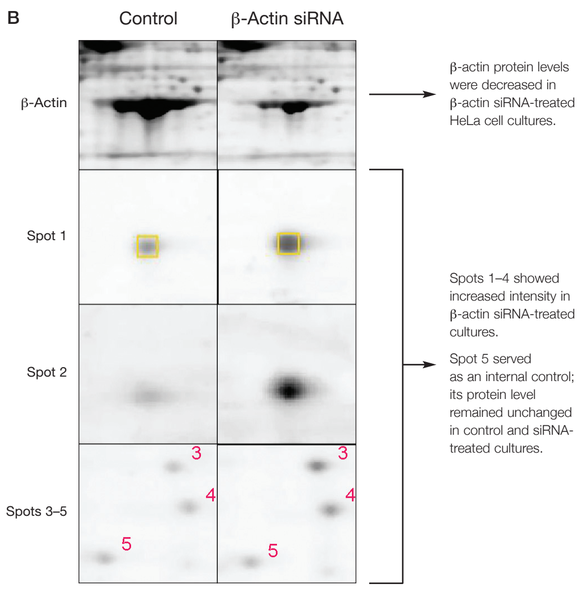


Fig. 2. Representative 2-DGE analysis of protein extracted from HeLa cells transfected with control or β-actin siRNA. A, 2-D gels showing total protein separation. The position of β-actin in both gels is indicated by the dotted box, and other proteins of interest are numbered; B, enlarged views of proteins of interest (β-actin and spots 1–4) and internal control (spot 5). Experiments were performed in triplicate, with two gels per treatment in each experiment.

Table. Protein identification. Spots were excised from 2-D gels and digested with trypsin. Peptide digests were analyzed by reverse-phase nanospray LC/MS/MS using an LTQ linear ion-trap mass spectrometer (Thermo Electron Corp.). Proteins were identified from MS/MS data by search of the human database using TurboSEQUEST with BioWorks 3.2 software (Thermo Electron Corp.). P = probability score; US = unified score.

Sample	P	US	Coverage (%)	Database Mass (Da)	Database pI	Identity
Control (β-actin)	1.9×10^{-13}	114.3	32.6	41,787	4.25	β-Actin
siRNA (β-actin)	2.7×10^{-12}	102.2	26.4	41,787	4.25	β-Actin
Spot 1	2.2×10^{-14}	70.4	36.1	18,492	8.33	Cofilin
Spot 2	2.2×10^{-8}	58.2	36.4	18,494	8.33	Destrin
Spot 3	1.6×10^{-12}	66.3	25.5	36,354	6.00	Annexin A3
Spot 4	1.2×10^{-9}	40.3	31.1	21,094	9.20	CAPZB
Spot 5	4.5×10^{-10}	38.3	18.8	29,787	4.25	Prohibitin

Cofilin, an actin depolymerization factor, binds to actin filaments and induces their cleavage (Hotulainen et al. 2005). It plays an important role in remodeling the highly dynamic structure of actin filaments (Maciver and Hussey 2002).

Destrin is also an actin depolymerization factor with a function similar to that of cofilin (Maciver and Hussey 2002, Ikeda et al. 2003).

Annexin A3, also known as lipocortin 3, is a calcium- and membrane-binding protein of unknown function that is located in early endosomes (Diakonova et al. 1997).

CAPZB is a subunit of the Cap Z protein, which is a widely distributed, highly conserved heterodimeric protein that binds to the barbed end of actin filaments but does not sever the filaments (Casella and Torres 1994).

Increases in the mRNA levels of cofilin, destrin, and CAPZB were observed in cells exposed to the β-actin siRNA. Annexin A3 mRNA levels were highly upregulated, probably as an artifact from the lipid-mediated transfection, since annexin is involved in endocytosis (Diakonova et al. 1997).

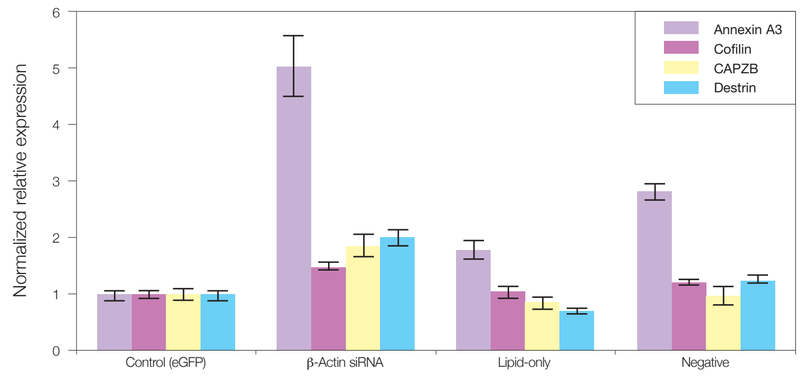


Fig. 3. RT-qPCR analysis of annexin A3, cofilin, CAPZB, and destrin mRNA levels. mRNA levels were measured by RT-qPCR using primers specific to each gene. Expression levels were normalized to those of glyceraldehyde-3-phosphate dehydrogenase (GAPDH) and 18S rRNA, and resulting relative expression values were rescaled to the normalized expression level of the sample transfected with control (eGFP) siRNA. Other controls for transfection efficiency included lipid-only transfections and transfection with a nonspecific control (negative) containing Opti-MEM buffer (Invitrogen Corp.).

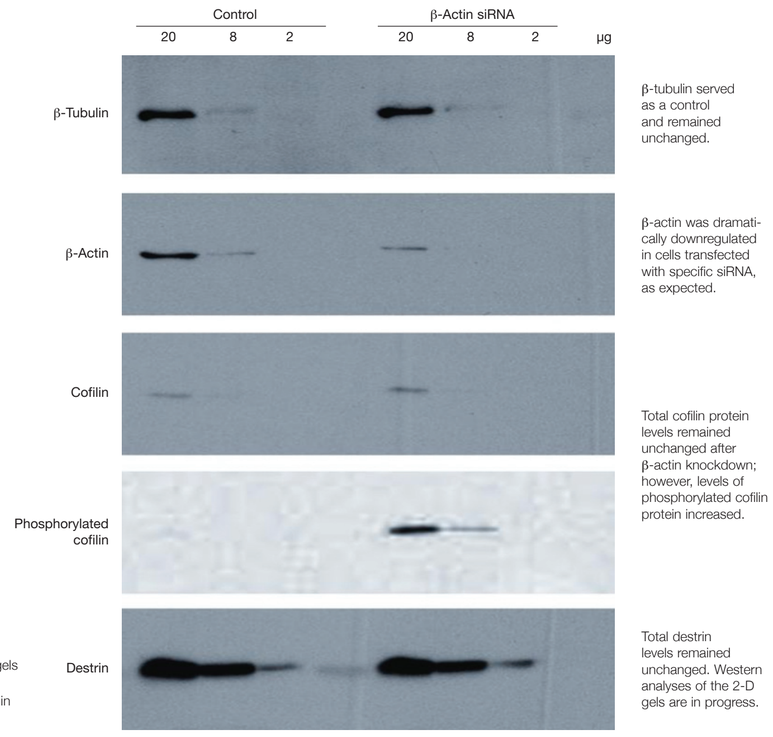


Fig. 4. Western blot analysis.

Conclusions

- siRNA-mediated knockdown of β-actin expression in HeLa cells induced changes in the levels of several proteins known to interact with actin filaments:
  - (Phosphorylated) cofilin — actin depolymerizing factor
  - Destrin — actin depolymerizing factor
  - CAPZB — actin filament capping protein
- Cofilin is activated by dephosphorylation (Gohla et al. 2005). Our study demonstrated that HeLa cells responded to β-actin knockdown by increasing levels of phosphorylated cofilin
- The combination of siRNA and 2-DGE technologies provides a powerful approach for discovering interacting proteins in a cellular pathway

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