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 directly or indirectly associated with actin filament function.

Experimental Workflow

Results

Table: Protein identification

<table>
<thead>
<tr>
<th>Sample</th>
<th>Protein Identification (%)</th>
<th>Mass (Da)</th>
<th>pI</th>
<th>Identity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td></td>
<td>50 – 75</td>
<td>37 – 37</td>
<td>70 – 75</td>
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<tr>
<td>β-actin siRNA</td>
<td></td>
<td>50 – 75</td>
<td>37 – 37</td>
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Fig. 2. Representative 2-DGE analysis of protein extracted from HeLa cells transfected with control or β-actin-specific 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: spots 1–4 and internal control (spot 5). Experiments were performed in triplicate, with two gels per treatment in each experiment.

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

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

Destrin is also an actin depolymerization factor with a function similar to that of cofilin (Macier and Hussey, 2002; Jedd et al., 2003). Annexin A3, also known as Annexin 3, is a calcium- and membrane-binding protein of unknown function that is located at cell membranes (Stasiak et al., 1998).

CAP23 is a subunit of the Cap23 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 Tomke, 1994).

Fig. 4. Representative 2-DGE analysis of protein extracted from HeLa cells transfected with control or β-actin-specific 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: spots 1–4 and internal control (spot 5). Experiments were performed in triplicate, with two gels per treatment in each experiment.

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Fig. 3. RT-qPCR analysis of annexin A3, cofilin, CAP23, and actin mRNA levels. β-Actin mRNA levels were normalized to the expression of glyceraldehyde-3-phosphate dehydrogenase (GAPDH) mRNA. Results are shown as mean ± SD. All experiments included control samples (no siRNA or transfection).