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Different delivery methods—different expression profiles

To the editor: Recent publications by several groups of researchers have suggested that small interfering RNAs (siRNA) delivered by lipid-mediated transfection induce both sequence-specific effects[1] and broad, class-specific changes in gene expression[1–4]. These findings challenge convictions previously held in the RNA interference (RNAi) community that assert virtual sequence specificity of siRNA knockdown, and they bring into question the value of this methodology as a research and therapeutic tool.

To test the individual contributions of siRNA and lipid delivery reagents to nonspecific gene modulation, we have compared gene-expression profiles in cells in which one of three different siRNAs targeting cyclophilin B (cyclo 1) or mitogen-activated protein kinase kinase 1, also known as MEK1 (MEK1-2 and MEK1-4), were delivered using Lipofectamine 2000 (L2K) or by electroporation. Our study demonstrates that even though the extent of target knockdown was similar for both modes of siRNA delivery (**Supplementary Fig. 1** online), the number of genes modulated by each method differed markedly (**Supplementary Fig. 2** online). In cells transfected with siRNA using L2K as a delivery vehicle (and using cells treated with lipid only as a reference control), 65 genes were consistently upregulated by more than 1.5-fold in all three samples (**Fig. 1a** and **Supplementary Table 1** online), thus mimicking the broad, class-specific gene modulation described previously[1–4]. Subtracting the values for the lipid-only reference during the analysis did not cancel out the observed upregulation of these genes. Thus, the data could be explained by a nonspecific response induced by siRNAs, an siRNA enhancement of lipid-induced effects, or a combination of the two.

The relative contribution of siRNAs and lipids to nonspecific gene regulation became evident when siRNAs were introduced into cells by lipid-independent methods. Microarray studies performed on HeLa cells electroporated with the same set of siRNAs (electroporation reference control) showed 1.5-fold upregulation of only 11 genes that were unrelated to the class modulated in samples treated with siRNA and lipid. This finding suggests that most of the RNAi-independent changes in gene expression observed during lipid-mediated transfection were the consequence of the delivery method that was used and not of the action of the siRNA.

Further studies in which cells were exposed to increasing concentrations of lipids in the absence of siRNA showed that 36 of the 65 genes that were upregulated in lipid-siRNA samples were again modulated, suggesting that this effect can be attributed to lipids (**Fig. 1b** and **Supplementary Table 2** online). Although it is possible that the remaining 29 genes were upregulated by the combination of L2K and siRNA, we believe it is highly unlikely that these data represent the effects of siRNA alone, as there were no genes commonly up- or downregulated by both lipofection and electroporation (**Supplementary Table 1**). Given these findings, we propose that nucleic acids alter or enhance the properties of lipid packaging reagents. If this is the case, future studies should consider more closely the contributions that delivery reagents make to alterations in gene expression and other phenotypic changes.

Array data are available online (http://www.dharmacon.com/tech/publications).

Note: Supplementary information is available on the Nature Methods website.

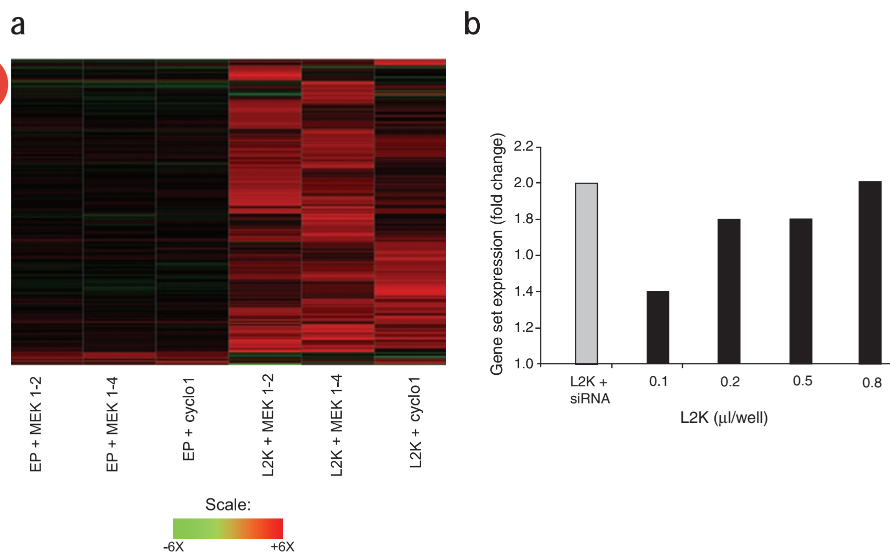

Figure 1 | Comparative microarray profiling identifies lipid carrier–mediated gene modulation. (**a**) Side-by-side comparison of upregulated gene expression heatmaps generated from cells transfected either with lipid and each of the three siRNAs (L2K, hybridized against mock-treated (lipid-only) cells) or electroporated with each of the three siRNAs (EP, hybridized against mock-treated (electroporated only) cells). (**b**) Average modulation of 36 genes upregulated upon L2K and siRNA treatment by increasing concentration of L2K alone. Gray bar represents gene modulation in cells treated with L2K (0.3 µl/well)and siRNA complex; black bars, gene modulation in cells treated with indicated amounts of L2K alone.

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Corrigendum: Different delivery methods—different expression profiles

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Supplementary Methods online of the Correspondence: in the "Lipofectamine 2000 Transfection" description, "10^3 cells/well" should read "2×10^4 cells/well".

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