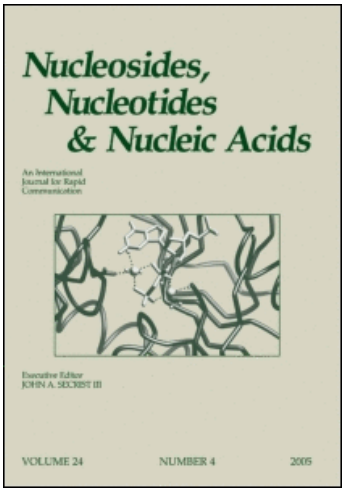

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Down-Regulation of Human Deoxyuridine Triphosphate Nucleotidohydrolase (dUTPase) Using Small Interfering RNA (siRNA)

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Down–Regulation of Human Deoxyuridine Triphosphate Nucleotidohydrolase (dUTPase) Using Small Interfering RNA (siRNA)

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ABSTRACT

A small interfering double stranded RNA molecule (siRNA, 21 bp) corresponding to a portion (nucleotides 337 to 357) of domain 3 of the human dUTPase was synthesized and used to determine whether it could down-regulate dUTPase activity in human cells. Transfection of the siRNA into HeLa and HT29 cells resulted in a 56 ± 3.6% decrease in dUTPase activity, while transfection of SW620 cells resulted in a 27 ± 6% decrease in dUTPase activity when compared to non-treated controls.

Key Words: siRNA; dUTP; dUTPase.

INTRODUCTION

It has been proposed that deoxyuridine triphosphate nucleotidohydrolase (dUTPase; EC 3.6.1.23) may be a potential target for the development of specific agents that could be useful for the treatment of infections caused by several microorganisms and also in cancer chemotherapy. However, studies on dUTPase in human cells have been hampered because cells deficient in dUTPase activity have not been isolated or constructed.

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The purpose of this study was to determine whether dUTPase activity could be down regulated using siRNA technology.

MATERIALS AND METHODS

HeLa, SW620 and HT29 cells were grown in McCoy's 5A medium supplemented with 10% (v/v) fetal calf serum (FCS; Intvitrogen-Gibco), penicillin (100 units/ml) and streptomycin (100 μg/ml) at 37°C in a 5% CO_2 atmosphere. Double stranded RNA (21 bp) targeted against a DNA sequence (5′-AAGATT ATAGAGGAAATGTTG; nucleotides 337–357) was synthesized by Xeragon (Germantown, MD) and transfected into the various cells using TransMessenger Transfection reagent (Qiagen) as described by the manufacturer. Briefly, cells (70–80% confluent, 0.8 to 1×10^6) were transfected in serum free medium for 4 hrs at 37°C in a 5% CO_2 atmosphere. Cells were washed twice and fresh medium containing 10% FCS and antibiotics was added. Forty-eight hours following transfection, cells were harvested, resuspended in a general extraction buffer (10 mM Tris-HCl, pH 7.5, 2 mM $MgCl_2$, 1 mM 2-mercaptoethanol and 20% (v/v) glycerol), lysed by sonication and centrifuged at 4°C for 5 min at 14,000 × g. The supernatants were used immediately for the determination of dUTPase and uracil-DNA glycosylase activities using procedures that we have described previously.[1] Protein was estimated using the Coomassie Blue procedure (BioRad) with bovine serum albumin as the standard.

RESULTS

Transfection of SW620, HT29 and HeLa cells with siRNA that targeted motif 3 of the human dUTPase resulted in decrease in dUTPase activity in the transfected cells

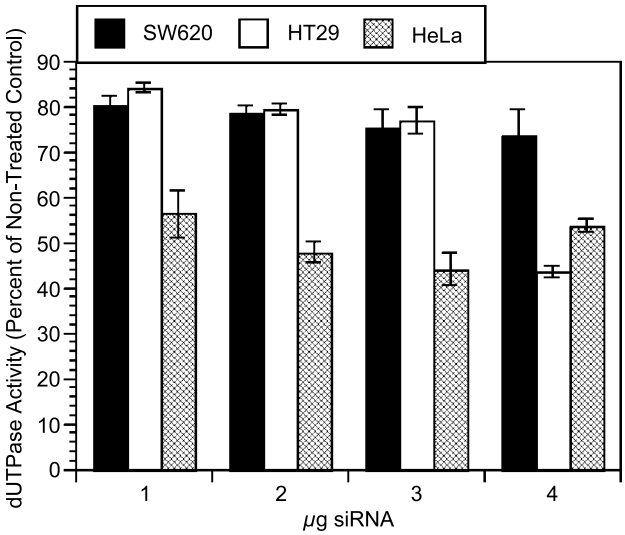

Figure 1. Effect of siRNAdUTP3 on dUTPase activity.

when compared to non-treated controls (Fig 1). The greatest decrease (56 ± 3.6%) in dUTPase activity occurred in HeLa cells transfected with 3 μg of siRNAdUTP3 and in HT29 cells (56 ± 1.4%) transfected with 4 μg siRNAdUT3. Conversely, transfection of SW620 cells with 4 μg of siRNAdUT3 resulted in only a 27 ± 6% reduction in activity.

Cells were transfected as described in Materials and Methods and examined for residual dUTPase activity. Results are expressed as the percent of dUTPase activity in treated cells when compared to non-treated controls. dUTPase activity (units/mg protein) in non-treated controls: SW620, 23.22 ± 1.65; HT29, 5.98 ± 0.78; HeLa, 18.67 ± 3.38.

DISCUSSION

While several compounds have been developed that inhibit purified dUTPases,[2–4] none of them have been shown to selectively inhibit dUTPase in cultured cells or in vivo. While structural data has been obtained for the human dUTPase, we do not believe that at this time it is possible to develop nucleotide analogs that would effectively inhibit the activities of this enzyme in vivo. This is because these nucleoside analogs would have to be delivered to cells as nucleosides and be metabolically activated. The intermediates formed during metabolic activation may function as substrates for several enzymes, including dUTPase. The complexities of such reactions make accurate analysis and interpretation of the data difficult if not impossible. Furthermore, since dUTPase is essential for the replication of *Escherichia coli*[5] and *Saccharomyces cerevisiae*,[6] it might not be possible to determine the precise roles of dUTPase in modulating the chemotherapeutic effectiveness of various compounds by the construction of dUTPase deficient cells lines and/or knock-out mice, Therefore, alternative approaches are required to determine whether the dUTPase can be used as a potential chemotherapeutic target.

The results of this study demonstrated that dUTPase activity was specifically down regulated in cells transfected with siRNA that targeted domain 3 of the human enzyme.

While the level of dUTPase inhibition differed between the cell lines, this probably reflects differences in the expression of dUTPase specific mRNA, differences in the stability of dUTPase and differences in transfection efficiencies of the cells or a combination of the above. These results demonstrate that a siRNA approach can be used to decrease expression of dUTPase in human cells.

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