Suppression of *BCR-ABL* mRNA by various ribozymes in HeLa cells

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ABSTRACT

Ribozymes are RNA molecules with enzymatic activity that can cleave target RNA molecules in a sequence specific manner. To date, various types of ribozyme have been constructed to cleave other RNAs and such *trans*-acting ribozymes include hammerhead, hairpin and HDV ribozymes. External guide sequence (EGS) can also induce the suppression of a gene-expression by taking advantage of cellular RNase P. Here we compared the activities of various functional RNA cleavers both *in vitro* and *in vivo*. The first purpose of this comparison was intended to determine the best ribozyme motif with the highest activity in cells. The second purpose is to know the correlation between the activities of ribozymes *in vitro* and *in vivo*. Our results indicated that the intrinsic cleavage activity of ribozymes is not the sole determinant that is responsible for the activity of a ribozyme in cultured cells.

INTRODUCTION

Several types of ribozymes that possess self-cleavage activities have been found in nature (1). Small-sized ribozymes that can be designed to cleave RNA strand intermolecularly include hammerhead, hairpin and HDV ribozymes (2-5). These *trans*-acting ribozymes recognize the substrate RNA via base pairings and then cleave the target RNA in a sequence specific manner. Because of their specificity, *trans*-acting ribozymes show promise to down-regulate a target RNA species.

The constitutive expression of the ribozyme *in vivo* under the control of a strong promoter is attractive for the application of *trans*-acting ribozymes to the gene therapy. As described in our previous reports (4-6), we succeeded in establishing an effective ribozyme-expression system based on an RNA Polymerase III promoter. High level expression under the control of the pol III promoter would be advantageous for ribozymes. Therefore, we chose the expression system with the promoter of a human gene for tRNA (4-6).
Another advantage to use tRNA-expression system is that it is possible to colocalize the expressed ribozymes with their target mRNAs. Ribozymes expressed under the control of tRNA promoters are exported to the cytoplasm as effectively as natural tRNAs. Similarly, the matured mRNAs are exported to the cytoplasm for the translation, so that both the ribozymes and the target mRNAs can co-locate in the same compartment.

On the contrary, the external guide sequence (EGS) is known to exert the functional activity in the nucleus (7). The EGS RNA binds to the target RNA mimicking the pre-tRNA structure that is recognized as a substrate by RNase P. Because RNase P which processes the 5'-portion of the immature tRNA exists in cells, for the use of the EGS as a gene-inactivating agent, it is not necessary to express additional RNase P.

Although these functional RNAs have recognized to be effective gene-inactivating agents, it remains unknown which ribozyme(s) can cleave the target RNA most effectively in mammalian cells. Herein we designed several types of functional RNAs targeted to the junction site of the BCR-ABL chimeric mRNA that causes the chronic myelogenous leukemia (CML) and we compared the intracellular activities of these catalytic RNAs with their cleavage activities in vitro.

RESULTS AND DISCUSSION

CML occurs by the reciprocal chromosomal translocations. They result in the formation of the BCR-ABL fusion gene; one of the chimeric mRNAs transcribed from the abnormal genes is the b2a2 mRNA (consisting of BCR exon 2 and ABL exon 2). We targeted the b2a2 mRNA and normal abl mRNA for the assay of functional RNAs.

We used six kinds of functional RNAs including hammerhead, hairpin and HDV ribozymes; maxizyme, minizyme and EGS. This minizyme is a minimized hammerhead ribozyme, with relatively high activity, found recently by means of in vitro selection (8). They were shortened at the common stem II region of a hammerhead ribozyme. Each ribozyme requires different specific sequence at the cleavage site, for example hammerhead ribozyme prefers the GUC sequence for the cleavage. Ribozymes expressed under the control of tRNAVal promoter or the U6 promoter, both of them belong to the pol III system.

The activities of the functional RNAs in vitro were measured under the condition of ribozyme excess. Ribozymes and substrate RNAs were synthesized by using T7 RNA polymerase. RNAs, with the length of 92mer and 121mer, corresponding to the regions adjacent to the junction of abl and b2a2 RNA, respectively, were used as substrates. The activities of the functional RNAs in HeLa cells were measured by using reporter constructs. The plasmids expressing each functional RNA were cotransfected with the target gene-expression plasmid that encodes b2a2- or abl-luciferase chimeric gene into the cultured cells. After the incubation, the luciferase activities in cells were detected by chemiluminescence. The decrease in the luciferase activity means that the functional RNAs suppressed the expression of the luciferase gene in cells.

Each functional RNA showed the cleavage activity both in vitro and in vivo but with different extents. We found no correlation between the tendencies of the ribozyme activities in vitro and in vivo. We further investigated the correlation of the localization of the ribozyme transcripts with the ribozyme activities in cells. We confirmed that, in order to maintain ribozyme activity in cells, the ribozyme transcripts should be localized in the cytoplasm and the EGS transcripts in mammalian cells, should be localized in the nucleus to cooperate with the endogenous RNase P.

REFERENCES