Development of Bioluminescent Probes for Visualizing and Quantifying of Endogenous RNAs in real time

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Gene expression plays important roles in physiological and pathophysiological phenotypic changes such as tumorigenesis, differentiation, and reprogramming. Temporal gene expression alteration includes important clues for comprehension of the mechanisms in the phenotypic changes. However, quantification of temporal changes in specific gene expression has not achieved in living samples at single cell resolution. In this study, we aim to develop probes for temporal monitoring of a specific RNA as the target gene expression product in single living cells by luminescence observation. We designed a luminescence-based RNA probe which consists of two fusion proteins with each different domain; One is an RNA recognition domain that binds to a specific RNA sequence, and the other is a split luciferase domain. The probe emits a luminescence signal through luciferase reconstitution by responding to the presence of target RNAs. (Fig. A).

Firstly, luminescence measurement was performed on living cells expressing the probes. The cells harboring the target RNA showed stronger luminescence in comparison to those without the RNA, indicative the target RNA-specific response of the probe. To assess the reversible reaction of the probe to target RNAs, we monitored luminescence of purified probe solution. The luminescence of the probe was weak in the absence of the target RNA. Upon addition of the RNA, the luminescence intensity increased, and following RNase treatment of the sample decreased the luminescence. Thus, the probe worked reversibly upon addition and digestion of target RNA. The time lapse luminescence images of living cells expressing the probes and target RNA were acquired under a luminescence microscope. The luminescence images were observed over several minutes at single cell resolution (Fig. B). We also prepared plasmid DNAs the coding region of which includes the gene of fluorescent protein mCherry fused with the target RNA sequence, and therefore the cells expressing the targe RNA can be identified by mCherry fluorescence. We introduce the plasmd into cultured cells together with the probe plasmid. The images of single-cell time-lapse observation showed that a substantial positive correlation between the mCherry fluorescence and the luminescence intensities. The time-lapse images suggest the potential of the probe to detect and quantify the target RNAs in single living cells.

These results totally suggest that the probes have potential to monitor target RNA abundance in single living cells over time. The RNA probes would provide a tool to monitor gene expression alteration for compreh ension of the mechanisms of the phenotypic changes.