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組織中の外来遺伝子発現の測定を目的とした治療外来遺伝子モニタリング

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【Objective】Efficiencies of transgene expressions in individuals are often variable in both viral and non-viral vectors. In clinical use, it is necessary to assess whether transgene expression is in the therapeutic range; however, monitoring of transgene expression in tissues has a difficulty, i.e., necessity of biopsy except for secretory proteins. In this study, we developed a novel method, named 'therapeutic transgene monitoring (TTM)', to monitor transgene expression in tissues by simultaneous administration of monitor gene.

【Methods】Plasmid DNA (pDNA) encoding firefly luciferease (Fluc) or secretory form of Gaussia princeps luciferase (Gluc) were used. Both pDNAs were administered into muscle or instilled onto gastric serosal surface in mice. Both luciferase activities in tissues and plasma were determined using luciferase substrates (luciferin for Fluc and coelenterazine for Gluc, respectively) and luminometer. Fluorescein- or rhodamine-labeled pDNAs were prepared using Label IT labeling kit (Mirus).

【Results and discussion】Good positive correlation was found between log-transgene expressions of reporter gene (Fluc) assuming therapeutic gene and monitor gene (Gluc) in the administered muscle after naked pDNA transfer into the muscle in mice. Also, log-transgene expressions of monitor gene in the administered muscle and plasma were positively correlated. As a consequence, positive correlation was evident between log-transgene expression of reporter gene in the muscle and that of monitor gene in the plasma. Positive correlations between log-transgene expression of reporter gene in the muscle and that of monitor gene in the plasma were also found in gastric serosal surface instillation of naked pDNA. Co-localization of two different fluorescently-labeled pDNA outside and inside of nucleus in gastric mesothelial cells could explain the good correlation. TTM will contribute to the safety of gene therapy.