# Efficient Expression of hG-CSF cDNA from an IRES-Dependent Bicistronic Vector Targeted to Mammary Gland of Transgenic Mice

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Previously, we observed high level expression of goat β-casein/genomic hGH fusion gene in mammary gland of transgenic mice. To develop an expression vector to make a human granulocyte-colony stimulating factor (hG-CSF) protein efficiently produced in milk of transgenic animals, we designed a new bicistronic vector using the goat β-casein/genomic hGH fusion gene as regulation sequences for expression and internal ribosome entry site (IRES) as a mediator for second gene expression. This vector was constructed by insertion of encephalomyocarditis virus (EMCV) IRES-dependent second gene region coupled with hG-CSF cDNA into 3' untranslated region of an intact hGH gene. By microinjcetion, four transgenic mice were generated and three of them transmitted the bicistronic vector to their progeny. A goat β-casein/genomic hG-CSF fusion gene was also constructed and microinjected for production of transgenic mice. In mammary gland of lactating transgenic mice carrying a bicistronic vector, both hGH gene and hG-CSF gene were transcribed as a single mRNA driven by goat β-casein promoter. The amounts of mRNA were comparable with that of endogenous mouse WAP mRNA. This result revealed that EMCV IRES sequences have minimal consequences for RNA processing in mammary gland of transgenic mice. In milk, hGH expression levels of first gene was detected at levels of 0.1, 2.4, and 9.0 mg/ml, whereas those of hG-CSF of IRES-mediated second gene were detected at levels of 10, 112, and 237 $\mu$g/ml, respectively. These levels of hG-CSF expression were also observed to be expressed up to 115 $\mu$g/ml from goat β-casein/genomic hG-CSF transgenic mice, implying that lower production of IRES-dependent hG-CSF than that of first hGH gene was not attribute to low efficiency of IRES-mediated translation. Taken together, these results demonstrate that a bicistronic vector using goat β-casein/hGH fusion gene and EMCV IRES sequences is available for efficient production of human protein in milk of transgenic animal.

Key words) *goat β-casein promoter, hG-CSF, EMCV IRES, transgenic mice*