

Fig. 4. Lipofection with Lipofectin (LPF) Combined with PGG in Colon 26

Colon 26 cells were transfected with LPF and PGG together. Lipofection and detection of EGFP used the same methods as for DC2.4 cells (*cf.* Fig. 2). (A) Transfected colon 26 with 2 μ g pEGFP-N1 vector only (without Lipofection) (upper left), vector+200 nM PGG (upper right), vector+LPF (lower left), vector+LPF+200 nM PGG (lower right). Small squares show images under phase-contrast microscopy. Scale bar=100 μ m. (B) EGFP gene transduction of colon 26 lipofected with the indicated combination was expressed as EGFP expression (EGFP-positive cell proportion (%)). Data are presented as the means \pm S.D. of two or three independent experiments. *** $p < 0.005$, vs. respective LPF-untreated group by two-tail unpaired Student's *t*-test. n.s. = no significant difference.

these cell surface receptors, the lipoplex/PGG complex may be easily phagocytosed. In previous studies, Li *et al.* reported that PGG binds to cell surface insulin receptor⁵¹⁾ and a number of researchers have studied the binding activity to biomolecules of PGG²⁹⁾; however it remains to be determined whether PGG binds to a DC-specific cell surface receptor. Furthermore, we have already suggested the importance of lipoplex incubation in combination with PGG for effective lipofection (data not shown). Consequently, we need to investigate the binding activity to cell surface receptor on DC2.4 cells and BMDCs in our further studies.

Secondly, we should focus on the DC maturation state if the action mechanism depends on the enhancement of phagocytosis. DCs have high phagocytic properties in the immature state, while few phagocytic properties in the mature state. Although DCs can phagocytose, they can be trans-

formed by lipofection only with very low efficacy, perhaps due to their high nuclease content or easy of maturity by transfection. In this study, we used an immortalized murine immature DC line, DC2.4 cells²¹⁾ and primary immature BMDCs. These cells may be matured by lipofection and lose phagocytic activity. As one possibility, PGG could maintain the phagocytic activity by inhibiting their maturation. DC maturation and differentiation are regulated by nuclear factor κ B 2 (NF- κ B2) and RelB, and these proteins are involved in vesicular transport.^{52–54)} Because previous reports have shown the inhibitory effect of PGG on NF- κ B activity,^{29,55)} PGG probably maintains an immature state by inhibiting a transcriptional factor, such as NF- κ B. However, it has been reported that lipofectin alone or lipoplex could not induce maturation on DCs related to the cell surface expression of major histocompatibility (MHC) class I or II, CD40,

CD80, CD86, ICAM-I and IL-12 p40 expression,^{33,34,53)} and an inflammatory signal, LPS, was needed to change immature DCs into mature DCs.⁵⁶⁾ Our phenotypical analysis of DC2.4 cells correspondingly demonstrated that cell surface expressions of MHC class II and CD80 were hardly increased by lipofection but were not inhibited by PGG (data not shown). Thus, we considered that PGG did not suppress DC maturation to maintain phagocytic activity.

Our data showed that PGG affected phagocytosis and lipofection efficacy in DC2.4 cells but the action mechanism has not been clarified. Hereafter, we must elucidate the mechanism in detail to understand DC biology and structure for a more highly effective lipofection method. If these challenges are achieved, DC vaccine therapy will develop markedly. Conventional DC vaccine therapies have already succeeded to some extent. In addition to these conventional therapies, DC vaccine therapy is expected to be highly effective and to be refined by more efficient lipofection of cytokines,⁵⁷⁾ chemokines⁵⁸⁾ and tumor-associated antigens (TAAs) using PGG.


Herbal medicine and medicinal plants have been used against a lot of diseases all over the world and considered enormous chemical library. Actually, various compounds have been found in medicinal plants, for example; resveratrol in grapes and the red wine prepared from them.³²⁾ But, these reports showed only biological activities of medicinal plant-derived compounds. Our studies exhibit not only biological activities but also the pharmaceutical technological effectiveness as an application. Finally, our present findings provide an expectation that constituents from herbal plant enhance lipofection efficacy.

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