OVER-EXPRESSION OF MIR-574-5P INCREASES TUMOR GROWTH IN XENOGRAFTS OF HUMAN LUNG CANCER CELLS VIA PROSTAGLANDIN E2

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Nonsmall cell lung cancer(NSCLC), the most common form of lung cancer, accounts for 80% of all cases(Molina et al.2008). Prostaglandin(PG)E2, a prostanoid generated through the coordinated activity of cyclooxygenase(COX)-2 and microsomal prostaglandin E synthase-1 (mPGES-1)(Jakobsson et al.1999), plays a central role in the development of various types of cancer(including lung cancer) by modulating epithelial cell proliferation, apoptosis, migration, invasion, angiogenesis and immune escape(Wang & Dubois, 2006). Recently, a novel selective mPGES-1 inhibitor Compound III(CIII, a benzoimidazole), has been synthesized; it inhibits both murine and human recombinant mPGES-1 with marginal inhibitory effects on other prostanoid synthases(Leclerc et al.2013). CIII profoundly inhibits PGE2 biosynthesis in A549 cells, a human lung adenocarcinoma cell line, as well as in LPS-stimulated human whole blood, i.e. in the presence of plasma proteins(Leclerc et al. 2013). Dysregulated expression of microRNAs(miRNAs) has a role in the development and progression of lung cancer. Among them, serum levels of miRNA-574-5p have been proposed as a novel biomarker for early-stage NSCLC(Foss et al.2011).

In this study, we aimed to investigate whether miRNA-574-5p over-expression in A549 cells influences xenograft tumor growth in nude mice. The possible role of enhanced PGE2 biosynthesis in A549 cell growth in vivo was investigated by CIII administration.

We used A549 cells with stable overexpression of miR-574-5p(A549-miR-574-5p cells) obtained by the lentiviral particles Mission®lenti miR-574-5p and the control A549 cell line(Mission®lenti control). A549-miR-574-5p cells or A549 control cells were injected into the hind flanks of nude mice and tumor formation was monitored up to four weeks. Tumor weight was assessed at sacrifice. Mice injected with A549 control cells developed 11 tumors, while mice injected with A549-miR-574-5p cells developed 12 tumors, which grew in a time-dependent fashion. At day 28, tumor volume was 0.059+0.08(cm3,mean+SEM) in mice injected with A549 control cells and 0.138+0.096cm3 in mice injected with A549-miR-574-5p cells($p<0.05$), respectively. Tumor weight was significantly higher in A549-miR-574-5p- derived tumors(0.048+0.009gr) vs A549 control-derived tumors(0.017+0.005gr, $p<0.01$). We evaluated PGE2 systemic biosynthesis measuring the urinary levels of PGE-M(1□-hydroxy-9,15-dioxo-2,3,4,5-tetranor-prostane-1,20-dioic acid) in 24h urine collections. Urinary PGE-M levels were significantly higher in mice injected with A549-miR-574-5p cells than in mice injected with A549 control cells(2.8+0.36 vs 1.5+0.09ng/mg creatinine, respectively,$p<0.01$). CIII(50mg/kg, p.o. for four weeks) did not affect basal tumor growth of mice injected with A549 control cells, but it significantly reduced the increase in tumor growth in mice injected with A549-miR-574-5p cells. In fact, at day 28, tumor weight was significantly reduced by CIII only in mice injected with A549-miR-574-5p(40%, $p<0.05$). Moreover, CIII reduced PGE-M

levels only in mice injected with A549-miR-574-5p(36%, $p<0.05$). Finally, the expression of Ki-67(as proliferative index), VEGF(a pro-angiogenic marker) and CD40(an inflammatory marker) in A549-miR-574-5p-derived tumors were markedly reduced by CIII administration.

In conclusion, miR-574-5p overexpression in A549 cells promoted tumor growth in vivo via enhanced PGE2 biosynthesis which was dependent on mPGES-1 activity. mPGES-1 inhibitors may represent effective anti-cancer agents. Our findings suggest that miR-574-5p expression might be a useful biomarker to select NSCLC patients who will respond to the anticancer effects of PGE2 inhibitors.

References

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