MICRORNA-574-5P MODULATES MICROSOMAL PROSTAGLANDIN E SYNTHASE EXPRESSION AND PROSTAGLANDIN E2 BIOSYNTHESIS IN A549 HUMAN LUNG CARCINOMA CELLS

1)Contursi A. 2)Saul MJ. 3)Baumann I. 4)Dovizio M. 5)Tacconelli S. 6)Korotkova M. 7)Jakobsson PJ. 8)Suess B. 9)Steinhilber D. 10)Patrignani P.

G. d'Annunzio" University of Chieti

MicroRNAs (miRNAs), a class of small non-coding RNA, are central players in the development and progression of cancer, including lung cancer. MiRNA-574-5p promotes the migration and invasion of nonsmall cell lung cancer (NSCLC) cell lines (Zhou et al., 2016). Several lines of evidence show that NSCLC is characterized by an increased expression of microsomal prostaglandin E synthase (mPGES)-1 (Yoshimatsu et al., 2001) which catalyzes the production of PGE2. This prostanoid is involved in cancer development due to its role in proliferation, migration/invasion, angiogenesis, inhibition of apoptosis and promotion of immune system escape (Wang et al., 2010). We aimed to verify whether the overexpression of miRNA-574-5p in A549 human lung carcinoma cells influences the expression of mPGES-1 and the biosynthesis of PGE2 in response to the proinflammatory stimulus interleukin(IL)-1. The effect of the new selective mPGES-1 inhibitor compound CIII (a benzoimidazole) (Leclerc et al., 2013) on the biosynthesis of PGE2 and PGF2a was evaluated.

A549 cells with stable overexpression of miR-574-5p (A549 miR-574-5p o.e) or control A549 cells were obtained using lentivirus technology. A549 miR-574-5p o.e cells were characterized by a 33-fold increase in miR 574-5p expression versus control A549 cells. A549 miR-574-5p o.e cells were characterized by enhanced proliferation (using MTT Cell Proliferation Assay Kit) versus control A549 cells when cultured both in the absence and the presence of 15% FBS (373.7 ± 31.60 and 528.0 ± 44.65 proliferation % time 0h mean±SEM, n=10 **p<0,05 versus A549 cells or A549 miR-574-5p o.e). Western blot analysis showed that both two cell lines did not express cyclooxygenase (COX)-2 while mPGES-1 was detectable. In response to IL-1β (for 24 hours), COX-2 was comparably induced in both cell lines. In contrast, mPGES-1 was significantly enhanced only in A549 miR-574-5p o.e cells. The biosynthesis of PGE2 (assessed by immunoassay) were significantly increased in A549 miR-574-5p o.e cells versus control A549 cells (13.68±0.3635 pg/µg protein versus 6.03±0.23 pg/µg protein,mean±SEM, n=3 **p<0,01). In contrast, PGF2a levels were comparable in both cell lines (6.08±0,4359 pg/µg protein and 6.64± 0.19 pg/µg protein, respectively; mean±SEM, n=3 **p<0,01 versus A549 cells or A549 miR-574-5p o.e). Altogether the results of prostanoid biosynthesis suggest the role of mPGES-1 induction in enhanced production of PGE2 detected in A549 miR-574-5p o.e cells.

CIII compound caused a comparable concentration-dependent inhibition of PGE2 biosynthesis in A549 miR-574-5p o.e cells and A549 control cells [IC50: 9 and 12 M (95% confidence interval, CI, 4.9-17,3 and 7,9-20,3) respectively]. Reduction of PGE2 biosynthesis was associated with enhanced production of PGF2a thus supporting a selective inhibitory effect of CIII on mPGES-1. At 30 M of CIII, PGF2a levels were enhanced versus DMSO vehicle by 274±28 and 219±49%, in A549

miR-574-5p o.e cells and A549 control cells, respectively. In contrast, the selective COX-2 inhibitor rofecoxib (0.3 □M) caused a comparable inhibition of PGE2 and PGF2a in both cell lines.

In conclusion, miR-574-5p overexpression was associated with the enhanced PGE2 biosynthesis in human lung carcinoma cells via increased expression of mPGES-1. Pharmacological inhibition of mPGES-1 activity may represent an effective strategy to cause an antitumorigenic effect in lung cancer. However, the possible shift of PGH2 metabolism towards the promitogenic PGF2a might mitigate the efficacy of selective mPGES-1 inhibitors. Further studies will be performed to verify the antitumorigenic efficacy of compound CIII in an experimental animal model of lung cancer.

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