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Upregulation of COX-2 in the lung cancer promotes overexpression of multidrug resistance protein 4 (MRP4) via PGE_2-dependent pathway

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ABSTRACT

It is apparent that lung cancer is associated with inflammation, with accompanying hallmark elevations of cyclooxygenase 2 (COX-2) and prostaglandin E2 (PGE_2) levels. However, the effects of these changes on MRP efflux transporters have not been thoroughly investigated before. Here, we report that upregulation of COX-2 can induce overexpression of MRP4 in both A549 non-small-cell lung cancer cell lines and mouse lung cancer models. In A549 cells, phorbol 12-myristate 13-acetate (PMA) treatment induced upregulation of COX-2 and MRP4 together, but not other MRP transporters. Transient overexpression of human COX-2 cDNA also specifically increased COX-2 and MRP4. Moreover, COX inhibitor treatment and COX-2-specific siRNA significantly inhibited the upregulation of MRP4. Additionally, PMA-treatment increased extracellular PGE_2 levels, likely due to increased MRP4 function. Likewise, COX-2-specific siRNA reduced extracellular PGE_2 levels. Furthermore, COX-2 upregulation resulted in an increase in mPGES-1, an enzyme responsible for PGE_2 production. Finally, metastasized lung cancer model mice exhibited increased expression levels of COX-2 and MRP4, as well as mPGES-1. In conclusion, the present study suggests that overexpression of MRP4 in lung cancer may be attributable to COX-2 upregulation via a PGE_2-dependent pathway.

1. Introduction

Lung cancer is one of the most life-threatening diseases in men and women worldwide. Although conventional chemotherapy plays a major role in the treatment of lung cancer, it is unfortunate that such chemotherapy often fails. For example, non-small-cell lung cancer tends to be intrinsically resistant to multiple anti-cancer agents, a phenomenon known as multidrug resistance (MDR). Although numerous transporters, such as P-glycoprotein (P-gp), are involved in MDR (Scagliotti et al., 1999), it is clear that members of the multidrug resistance protein (MRP) family play an important role in MDR, too. Indeed, there is a previous report that MRP1, MRP2, and MRP3 are responsible for MDR in lung cancer (Young et al., 2001). However, few data are available for other MRPs, such as MRP4, in lung cancer.

Regarding MRP4's characteristics, Reid et al. (2003) first reported that MRP4 has unusual properties as an efflux transporter. Unlike other homologous MRP subfamilies, MRP4 transports endogenous small molecules such as cytosolic cyclic adenosine monophosphate (cAMP), cyclic guanosine monophosphate (cGMP), and prostaglandin E_2 (PGE_2) (Reid et al., 2003). MRP4 also transports a wide variety of drugs including antiviral, cytostatic, antibiotic, and cardiovascular drugs (Russel et al., 2008). Most importantly, MRP4 can expel some anti-cancer agents, such as methotrexate (van Aubel et al., 2002), topotecan (Leggas et al., 2004), and irinotecan (Tian et al., 2005), that are widely used in the treatment of various lung cancers (Kwong et al., 2002; Langer 2001). Thus, it is expected that MRP4 in lung cancer will likely worsen the sensitivity to these MRP4-substrate anti-cancer drugs.

Abbreviations: CAR, constitutive androstane receptor; cAMP, cytosolic cyclic adenosine monophosphate; cGMP, cyclic guanosine monophosphate; COX-2, cyclooxygenase 2; BCA, bicinchoninic acid; BCRP, breast cancer resistance protein; MDR, multi-drug resistance; MRP4, multidrug resistance associated protein 4; MRPs, multidrug resistance associated proteins; PGE_2, prostaglandin E2; PGES, prostaglandin E synthase; P-gp, P-glycoprotein; PMA, phorbol 12-myristate 13-acetate; VDR, vitamin D receptor.

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In particular, PGE_2, an endogenous substrate of MRP4, is an endogenous lipid that plays a critical role in inflammation and tumorigenesis, and is derived from arachidonic acid through the action of cyclooxygenase (COX). Generally, COX enzymes promote the production of prostaglandins (PGs), which have diverse roles in inflammation, cell proliferation, migration, and angiogenesis. Moreover, it seems that COX-2, an inducible COX isozyme, is tightly linked to PGE_2 production in various cancers, including lung cancer (Shaik et al., 2004). Accumulated PGE_2 is then transported out into the extracellular microenvironment through a transporter where it exerts various actions in tumorigenesis and cancer progression. As mentioned above, this transport is mediated primarily by MRP4 and the transport process is quite MRP4 specific. Indeed, it has been found that MRP1, MRP2, MRP3, and MRP5 are incapable of transporting PGE_2 (Reid et al., 2003).

Considering that the presence of substrates can regulate the expression and/or function of corresponding transporters, it is possible to hypothesize that PGE_2, an endogenous substrate of MRP4, may also be involved in the regulation of MRP4 expression. However, to our knowledge, no report has examined any relationship between COX-2/PGE_2 and MRP4, especially in lung cancer. Thus, in the present study, the possibility of changes in the expression level of MRP4 with regard to COX-2 and PGE_2 was investigated in a lung cancer cell line (A549 cells) and lung cancer-induced mice.

2. Methods

2.1. Materials

Phorbol 12-myristate 13-acetate (PMA), RPMI 1640 cell culture medium (powder with L-glutamine and without sodium bicarbonate), phosphate-buffered saline (PBS, pH 7.4, 0.01 M), and indomethacin were purchased from Sigma–Aldrich (St. Louis, MO). SC236 was purchased from Tocris (Ellisville, MO). Cay10526 was purchased from Cayman (Ann Arbor, MI). The human lung cancer cell line A549 and the murine colon cancer cell line CT-26 were purchased from the Korea Cell Line Bank (Seoul, Korea). All other reagents were obtained from Sigma–Aldrich (St. Louis, MO).

2.2. Cell culture and PMA treatment

A549 (human lung cancer) cells were grown routinely at 37 °C in RPMI 1640 supplemented with 10% fetal bovine serum (FBS), 100 IU/mL penicillin, and 100 mg/mL streptomycin in a 5% CO_2/95% air humidified atmosphere according to standard protocols (Maeng et al., 2014). At confluence, cells were harvested and seeded at a density of 1.5×10^5 cells per well. Two days after seeding, A549 cells were treated with 10 nM PMA, a well-known COX-2 inducer, for 6, 12, 24, 36, or 48 h. In the presence of PMA, various COX inhibitors including 30 μM indomethacin, 5 μM celecoxib, and 5 μM SC236 were co-added for 48 h.

2.3. Transfection of COX-2 cDNA or siRNA in A549 cells

A549 cells were seeded in 100-mm dishes at 1.0×10^6 cells/dish. On the second day, the cells were transfected following the manufacturer's protocol with human COX-2 cDNA or siRNAs using FuGene HD (Promega, Madison, WI) or lipofectamine2000 (Invitrogen, Carlsbad, CA), respectively. The COX-2-specific siRNA oligomers were designed and ordered from Bioneer (Daeduk, Korea). The siRNA sequences targeting human COX-2 are listed in Table 1. After the transfections, cells were allowed to grow for one more day, and experiments were then performed.

2.4. Lung cancer metastasis induction

Lung tumor-bearing mice were prepared as described previously (Yang et al., 2009). Briefly, 3–4-week-old male CDF_1 mice weighing about 18–22 g were purchased from Central Laboratory Animal, Inc. (Seoul, Korea). A transplantable mouse colon cancer cell line (CT-26), which was maintained in RPMI 1640 medium supplemented with 10% FBS, 100 IU/mL penicillin, 100 μg/mL streptomycin, and 2 mM L-glutamine in 5% CO_2 humidified air at 37 °C, was administered to the CDF_1 mice via the tail vein at 1×10^5 cells/mouse. The animals were then identified as carrying lung cancers 2 weeks after the injection (Yang et al., 2009). All animal experiments were performed according to the Guidelines for Animal Care and Use of Seoul National University, Seoul, Korea.

Table 1
Sequences of oligomers sets.

Experiment	Species	Gene	Forward sequence	Reverse sequence
PCR	Human	COX-2 (PTGS2)	GCAGTTGTTCCAGACAAGCA	CAGGATACAGCTCCACAGCA
		MRP4 (ABCC4)	GTCAGACCTGGCGAATTGTT	GTAAGTCCCCTTCTGCACCA
	Mouse	Cox-2 (Ptgs2)	CTGGTGCCTGGTCTGATGATG	GAGTCTGCTGGTTTGGAATAGTTG
		Mrp4 (Abcc4)	CCAGACTTTGCACAACAGGA	TTACAAAGATGGCGCAGATG
RT-qPCR	Human	GAPDH	GAAGTTGGAGGTCGGAGTC	GAAGATGGTGATGGGATTTC
		COX-1 (PTGS1)	TGGCTGGGCGTGCTAGAGGT	CAGCCTGCGTGAGGTGTGTC
		COX-2 (PTGS2)	GGAACACAACAGAGTATGCG	AAGGGGATGCCAGTGATAGA
		MRP1 (ABCC1)	TCTACCTCCTGTGGCTGAAT	CCGATTGTCTTTGCTCTTCA
		MRP2 (ABCC2)	TCCTTGCGCAGCTGGATTAC	TCGCTGAAGTGAGAGTAGAT
		MRP3 (ABCC3)	CAGAGAAGGTGCAGGTGACA	CTAAAGCAGCATAGACGCCC
		MRP4 (ABCC4)	TGATGAGCCGTATGTTTTGC	CTTCGGAACGGACTTGACAT
		MRP5 (ABCC5)	ACGGAAAGAGGCACCCATGA	TGTTCCCGCTTCCTTGCTTG
		mPGES-1 (PTGES1)	GCAAAGTGGTACGATCGAAGG	GAGTAGACGAAGCCCAGGAA
		mPGES-2 (PTGES2)	CTGCAGAAGGGACACGTCTT	AGGACCTCCACGCAGAGC
		cPGES (PTGES3)	GCAAAGTGGTACGATCGAAGG	TGTCCGTTCTTTTATGCTTGG
		15-PGDH (HPGD)	TGAATCCAACTATGCCATGC	GTGAAGGCGGCATCATTATC
	Mouse	Gapdh	AGGTCGGTGTGAACGGATTTG	TGTAGACCATGTAGTTGAGGTCA
		Cox-2 (Ptgs2)	TGCACTATGGTTACAAAAGCTGG	TCAGGAAGCTCCTTATTTCCCTT
		Mrp3 (Abcc3)	CTGGGTCCCCTGCATCTAC	GCCGTCTTGAGCCTGGATAAC
		Mrp4 (Abcc4)	CATCGCGGTAACCGTCCTC	CCGCAGTTTTACTCCGCAG
		mPges-1 (Ptges1)	CACACTGCTGGTCATCAAGAT	TCACTCCTGTAATACTGGAGGC
siRNA	Human	COX-2 (PTGS2)	CACCAAGAGTATAAACCTT	AAGGTTTATACTCTTGGTG
	–	Scrambled siRNA control	CCTACGCCACCAATTTCGT	ACGAAATTGGTGGCGTAGG