Graphical Review

Hydrogen sulfide and its donors: Novel antitumor and antimetastatic therapies for triple-negative breast cancer

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ABSTRACT

Hydrogen sulfide (H2S) is considered as a novel second-messenger molecule associated with the modulation of various physiological and pathological processes. In the field of antitumor research, endogenous H2S induces angiogenesis, accelerates the cell cycle and inhibits apoptosis, which results in promoting oncogenesis eventually. Interestingly, high concentrations of exogenous H2S liberated from donors suppress the growth of various tumors via inducing cellular acidification and modulating several signaling pathways involved in cell cycle regulation, proliferation, apoptosis and metastasis. The selective release of certain concentrations of H2S from H2S donors in the target has been considered as an alternative tumor therapy strategy. Triple-negative breast cancer (TNBC), an aggressive subtype with less than one year median survival time, is known to account for approximately 15–20% of all breast cancers. Due to the lack of approved targeted therapy, the clinical treatment of TNBC is still hindered by metastasis as well as recurrence. Significant efforts have been spent on developing novel treatments of TNBC, and remarkable progress in the control of TNBC by H2S donors and their derivatives have been made in recent years. This review summarizes various pathways involved in antitumor and antimetastasis effects of H2S donors and their derivatives on TNBC, which provides novel insights for TNBC treatment.

1. Introduction

H2S has been discovered as a novel endogenous signaling gaseous transmitter along with nitric oxide (NO) and carbon monoxide (CO) [1,2] since Abe and Kimura revealed the endogenous production and intracellular signaling cascades of H2S [3]. In general, taking cysteine as a substrate, endogenous H2S liberation is mainly attributed to cystathionine-β-synthase (CBS), cystathionine-β-lyase (CSE) and 3-mercaptopyruvate sulfurtransferase (3-MST) (Fig. 1) [4]. The liberated H2S participates in modulating physiological and pathological processes, including intestinal disorders [5], cardiovascular diseases [6–9], diseases caused by oxidative stress [10–12] and inflammation [13,14]. Sodium hydrosulfide (NaHS) and sodium sulfide (Na2S) were generally used as the H2S donors in early studies. Although sulphide inorganic salts exhibited cytotoxic effects on several tumor cells and therapeutic potential on the cardiovascular system [15–17], rapid oxidation of NaHS and Na2S solution and uncontrolled release of H2S lead to the discrepancies in curative effect [18]. Recently, some organic H2S donors (ADT-OH, thiobenzamide, GYY4137, DADS and DATS, Fig. 2) are used for endogenous H2S production in a sustained fashion [19–22]. Several H2S donor-based therapeutics have entered Phase II clinical trials, such as ATB-346 and GIC-1001 (Fig. 2) [23,24]. Besides cardiovascular diseases and chronic diseases [25–32], H2S donors show significantly different roles in neoplasia compared with endogenous H2S. Endogenous H2S and low levels of exogenous H2S induce angiogenesis, accelerate cell cycle, inhibit apoptosis, and promote oncogenesis eventually [33,34], whereas H2S donors trigger high concentrations of exogenous H2S production to prevent tumor development. The donors

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have been shown to inhibit NF-κB and IκB activities, which indicates cell migration inhibitory effects of HS-ASA (a novel H2S donating aspirin, Fig. 2) on TNBC MDA-MB-231 cells in vitro and in vivo. HS-ASA was shown to inhibit proliferation accompanied by cell cycle arrest at G0/G1 phase in a dose-dependent manner. It markedly restrained the translocation of NF-κB (p65) into the nucleus via inhibiting the phosphorylation status of IKKa and IKKβ to decrease dissociation between NF-κB and IkB. Furthermore, after HS-ASA treatment, thioredoxin reductase-1 (TrxR) inhibition induced reactive oxygen species (ROS) accumulation also disrupted redox homeostasis in MDA-MB-231 cells. The subsequent decline of tumor mass and volume in mouse xenograft model indicated potent antitumor effects of HS-ASA on TNBC through inhibiting NF-κB pathway and TrxR activity, and elevating ROS levels [62].

Besides eliminating tumors, Liu et al. demonstrated that DATS (an extract of garlic) resulted in sharp attenuation of MDA-MB-231 cell migration, and metastasis phenotype inhibition was observed in zebrafish xenograft model. After the treatment with DATS, MDA-MB-231 cells with smooth surfaces and decreasing pseudopodia were observed, which indicated cell migration inhibitory effect. In addition, by blocking the NF-κB pathway, DATS significantly inhibited the mRNA levels, protein expression, and enzyme activity of MMP-2/9, the proteases over-expressed during invasion, oncogenesis and metastasis, to degrade and reshape the dynamic balance of extracellular matrix. After DATS treatment, phosphorylation of ERK level was declined in a dose-dependent manner, which proved that the ERK/MAPK pathway was also associated with TNBC [63]. It has become a consensus that breast cancer stem cells play an important role in tumor proliferation and metastasis. As an important transcription regulator, the expression of FoxQ1 is related to the growth and metastasis of breast cancer. The down-regulation of FoxQ1 expression in DATS-treated SUM159 TNBC cells was discovered. As one of the markers of tumor stem cells, ALDH1 activity was completely inhibited after FoxQ1 knockdown and was further inhibited in SUM159 xenograft model. These studies showed that FoxQ1 might be a new target for TNBC treatment by DATS [64].

As a volatile extract of garlic oil, DADS also endowed with potent inhibitory effects on TNBC. Like DATS, Huang et al. confirmed the inhibitory effect of DADS on migration and invasion through wound-healing, transwell migration and invasion assays. The expression and activity of MMP-9 were significantly declined after the incubation with DADS. Besides, the potent antimetastatic effect of DADS was also revealed based on the observed reversal of the epithelial-mesenchymal transition (EMT). As an AU-rich RNA-binding protein, tristetraprolin (TTP) involves in the degradation of urckinase type plasminogen activator (uPA), an upstream gene of MMP-9, and inhibits tumor metastasis by mediating the expression of MMP-9. As reported by Xiong et al., decline of uPA and upregulation of TTP were detected after DADS treatment, which could be completely counteracted by TTP siRNA. Therefore, targeting TTP might be capable of exerting inhibitory effect on TNBC metastasis. By ameliorating aberrant activation of the β-catenin pathway, DADS improved the activity of caspase-3/9 and the expression of pro-apoptotic factor Bax, while it down-regulated anti-apoptotic factor Bcl-2 levels, protein expression, and enzyme activity of MMP-2/9, the proteases over-expressed during invasion, oncogenesis and metastasis, to degrade and reshape the dynamic balance of extracellular matrix. As an important transcription regulator, the expression of FoxQ1 is related to the growth and metastasis of breast cancer. The down-regulation of FoxQ1 expression in DATS-treated SUM159 TNBC cells was discovered. As one of the markers of tumor stem cells, ALDH1 activity was completely inhibited after FoxQ1 knockdown and was further inhibited in SUM159 xenograft model. These studies showed that FoxQ1 might be a new target for TNBC treatment by DATS [64].

ADT-OH, a commonly used H2S donor that exhibited prominent cytoprotective properties [67], was conjugated with hyaluronic acid (HA) to improve the retention time and water solubility. Similarly, Dong et al. found that HA-ADT (Fig. 2) induced MDA-MB-231 cells apoptosis by elevating the ratio of Bad/Bcl-xl and Bax/Bcl-2 as well as the expression of cleaved caspase-3/9 and cleaved PARP. Further preliminary mechanism studies found PI3K/Akt/mTOR and Ras/Raf/MEK/ERK signaling pathways were suppressed in HA-ADT group compared with the control (PBS), NaHS and GYY4137 groups. The markedly shrunken xenograft tumors were also found in HA-ADT-treated nude mice, accompanied with reduced CD31 expression that
demonstrated angiogenesis inhibition [68].

In summary, on the one hand, H2S donors and their derivatives effectively modulated the proliferation and apoptosis of TNBC cells by inhibiting the phosphorylation or expression of proteins associated with NF-κB, PI3K/Akt/mTOR and Ras/Raf/MEK/ERK signaling pathways. On the other hand, they also inhibited the expression of MMP-2/9 and EMT to resist TNBC metastasis by inhibiting aberrant activation of the β-catenin pathway. Other pathways that H2S may be involved in TNBC prevention deserve further investigation. The mechanism of H2S donors and their derivatives against TNBC is summarized in Fig. 3. As for sulfide salts, although NaHS administration alone exhibits little inhibitory effect on tumor growth in mice, it decreases O2 consumption and increases O2 delivery to alleviate tumor hypoxia, eventually radiosensitized MDA-MB-231 tumors. The results provided insights for H2S donors in combination therapy [69].

2. Conclusions and perspectives

Since the current therapies for TNBC are mainly adjuvant chemotherapy or radiotherapy after surgery, patients can hardly bear side effects of long-term chemotherapy or radiotherapy, and they still face the risks of metastasis and relapse due to the lack of targeted drug molecules. Recently, H2S donors and their derivatives have made attractive development in TNBC therapy. Although H2S donors liberate high concentrations of H2S, there is no research on actual impact of the H2S itself in TNBC treatment, and it is unclear whether the remaining structural fragments after H2S generation are related to the potent antitumor activity. The precise antitumor mechanism of H2S deserves further study. Moreover, it is worth noting that the regulation mechanism of the expression and activity of CBS, CSE and 3-MST is remained ambiguous and argued. Given the evidence that inhibition of CBS induces apoptosis in several tumor cells (colon cancer, ovarian cancer, breast cancer, etc.), perhaps inhibition of H2S biosynthesis through targeted inhibition of H2S-producing enzymes exhibits comparable antitumor effects to H2S donors. At present, investigations also elaborated on the connection between H2S and NO which participated in modulating multiple physiological and pathological processes [70,71], such second messenger molecules provide novel insights for TNBC treatment. In light of the bell-shaped model effect of H2S has been verified and widely accepted, the selective release of certain concentrations of H2S from H2S donors in the target to minimize the adverse effects of H2S is essential. To cope with the special tumor microenvironment, H2S donors that sensitive to definite pH, enzymes, NIR light or free radicals are being studied [72–75]. Nanosized delivery systems have also been tried for targeted delivery of exogenous H2S and hope to endow it with improved antitumor effect through intratumoral conversion of nanostructures to microstructures [76,77]. These attempts may help researchers to study the precise mechanisms of H2S in the therapy of cancers, especially TNBC. We hope that H2S donating compounds can greatly improve the survival rate of TNBC patients in
involved in NF-κB stimuli. They not only promote TNBC cells apoptosis by activating mitochondrial apoptosis pathway and inhibiting phosphorylation or expression of related proteins activity inhibition and EMT reversal.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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