Involvement of the L-Cysteine/Hydrogen sulphide pathway in human melanoma.

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The need for new drugs in melanoma treatment is of great relevance. Indeed, current therapies for the treatment of metastatic melanoma offer a limited clinical benefit and only in recent years there has been an advancement due to the identification of new molecular targets (Flaherty KT. et al., 2012). Hydrogen sulphide (H_2S) is endogenously produced by the action of three enzymes CBS, CSE and the newly discovered 3-MST (Wang R. 2012). While H_2S is cytoprotective at physiological concentrations, it seems to have pro-apoptotic actions in cancer cells (Predmore BL. et al., 2012). However, to date there are not definitive reports on the role played by H_2S in cancer development. Aim of this study was to determine the possible involvement of H_2S in human melanoma. The study has been performed by using some relevant human melanoma cell lines such as A375, WM115 and SK-Mel-28. Normal human epidermal melanocytes (NHEM) were used as control. Cellular proliferation was evaluated by the MTT assay. Apoptosis was assayed by flow cytometry analysis by double staining with Annexin V and propidium iodide (PI). NF-κB/DNA-binding activity was evaluated by electrophoretic mobility shift assay. Expression of CBS, CSE, 3-MST was assayed by quantitative real time RT-PCR, expression of Bcl-2, XIAP, c-FLIP, caspase 3, PARP, IKBα and Akt/p-Akt was determined by western blotting. Levels of H_2S in the supernatant and in total cellular extracts were assayed by colorimetric assay. Diallyl trisulfide (DATS) is a garlic-derived polysulfide able to release H_2S (Benavides GA. et al., 2007). Our results demonstrate that DATS greatly suppressed, in a time and concentration-dependent manner, proliferation of the three human melanoma cell lines used. The most striking effect was obtained on the A375 cell line whose proliferation was inhibited, following incubation with DATS (10-30-100 μM, 72h) by 30, 70, and 78% respectively ($p<0.001$). This effect well correlated with the significant increase in H_2S levels found in both supernatants and cellular lysates. Conversely, DATS up to 300 μM did not affect proliferation of NHEM. DATS-induced inhibition of A375 proliferation (10-100μM, 72h) was almost completely reversed by haemoglobin (10 μM; $p<0.001$) a scavenger of H_2S. Moreover, DATS-induced inhibition of A375 proliferation was due to the induction of apoptosis as demonstrated by FACS analysis with Annexin V/PI staining and further confirmed by the inhibition of Bcl-2, XIAP, FLIP, as well as by the cleavage and consequent activation of caspase-3 and inactivation of poly (ADP ribose) polymerase (PARP-1). Constitutive NF-κB, activated Akt and ERK 1/2 expression have been described in melanoma (Kantrow SM. et al., 2007). We also demonstrated that H_2S released by DATS suppressed both constitutive NF-κB/DNA-binding activity, Akt and ERK 1/2 phosphorylation suggesting that the apoptotic effect observed following exposure to H_2S was consistent with the signal transduction pathways activated. In conclusion, we demonstrate that H_2S triggers, in relevant human melanoma cell lines, an apoptotic effect. This effect, in turn, activates downstream a signal pattern that candidates H_2S as a possible novel therapeutic/target or diagnostic tool.

Flaherty KT et al. (2012). *Nat Rev Cancer.* 12: 349-61.
Wang R. (2012). *Physiol Rev.* 92: 791-896.
Predmore BL. et al. (2012). *Antioxid Redox Signal.* 17: 119-40.
Benavides GA. et al. (2007) *Proc Natl Acad Sci U S A.* 104: 17977-82.
Kantrow SM. et al. (2007) *J Cutan Pathol.* 34: 593-6.