S-Allylcysteine inhibited the growth of three-dimensional HepG2 liver spheroids and upregulated the gene expression of *ORS1*

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Hepatocellular Carcinoma (HCC) is one of the most prevalent malignant tumors and ranks second in terms of cancer-related death in males worldwide. Various curative strategies are proposed for HCC, which leads to serious side effects resulting from the antitumor drugs. HCC is a complicated biological process which involves various genetic and epigenetic parameters. *OSR1* (Odd-skipped related 1) is a tumor suppressor gene in several cancer cell lines and plays an important role in regulating cell survival and DNA methylation. *OSR1* expression was downregulated in cancer cell lines and it was associated with DNA hypermethylation. S-allylcysteine, a water-soluble garlic derivative, suppressed cell proliferation and decreased global DNA methylation levels in vitro. The aim of this study was to assess S-allylcysteine effects in a three-dimensional (3D) human hepatocellular carcinoma (HepG2) cells cultures such as a multi-cellular spheroids (MTS). MTS mimic tumor physiological conditions while promote cell-cell interaction. HepG2 spheroids were generated and maintained on a self-assemble ultra low attachment 96-well plates to evaluated the effects of S-allylcysteine (12.5 mM – 100 mM) on growth, morphological integrity and cell viability. We also evaluated the expression of *OSR1* and genes related to DNA methylation and histone acetyltransferase (*DNMT1, DNMT3A, DNMT3B* and *HAT1*). S-allylcysteine significantly decrease the viability of MTS. Notably, decreased cell viability in MTS caused by S-allylcysteina (50 or 100 mM) was detectable using resazurin assay. HepG2 MTS were cultured and imaged over the time of growth. Phase contrast

microscope and software analysis were employed to describe the growth kinetics and the MTS volume. After three days of treatments, MTS at two concentrations (25 and 50 mM) of S-allylcysteine showed volume reduction. MTS also were exposed for three days to two concentrations (50 and 100 mM) of S-allylcysteine, and the expression of three genes of the DNA methylation pathway (*DNMT1, DNMT3A* and *DNMT3B*) was downregulated, while *OSR1* expression was significantly upregulated at both concentrations. According to literature, *OSR1* gene encoding a zinc-finger transcription factor and its expression was frequently silenced by hypermethylation in cancer cell lines. Since the genes responsible for DNA methylation were downregulated, it is possible that hypermethylation of the *OSR1* gene did not occur. Thus, the upregulated expression of *OSR1* after treatments with S-allylcysteine may have contributed to the reduction of cell viability and volume of HepG2 MTS. In conclusion, *OSR1* transcriptional activation and methylation levels may serve as a potential therapeutic strategy for improved management of HCC.

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