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Reversion from Methionine Addiction to Methionine Independence Results in Loss of Tumorigenic Potential of Highly-malignant Lung-cancer Cells

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Abstract. *Background/Aim: Methionine addiction, a fundamental and general hallmark of cancer, is due to the excess use of methionine for transmethylation, and is described as the Hoffman-effect. Methionine-addicted cancer cells can revert at low frequency to methionine independence when selected under methionine-restriction. We report here that highly-malignant methionine-addicted H460 human lung-cancer cells, when selected for methionine independence, have greatly-reduced tumorigenic potential. Materials and Methods: Methionine-addicted H460 parental cancer cells and methionine-independent revertant H460-R1 cells were injected in nude mice subcutaneously. Results: When the parental H460 methionine-addicted cells were injected in nude mice at 2.5×10^5, 1×10^5 and 5×10^4, the cells could form tumors. In contrast, the H460-R1 methionine-independent revertant cells could not form tumors when the above-listed cell numbers were injected in nude mice. Conclusion: There is a tight linkage between methionine addiction and malignancy.*

Methionine addiction, first discovered by one of us (RMH) almost half a century ago (1), is a fundamental and general hallmark of cancer. Methionine addiction is due to excess use of methionine for transmethylation reactions (2-6), which is termed the Hoffman-effect (7). The excess transmethylation in cancer cells is due, at least in part, to excess methylation of histone H3 lysine marks (8-11). Excess histone H3 lysine methylation has been shown to be associated with malignancy, since it is not present in normal cells or methionine-independent revertants isolated from the methionine-addicted cancer cells (10, 11). Under methionine restriction effected by culturing cells in methionine-free medium, or putting tumor-bearing mice on a low-methionine diet, or in the presence of recombinant methioninase, methionine-addicted cancer cells arrest in late S/G_2 phase of the cell cycle (12-14) due to unstable methylation of histone H3 lysine marks (10).

Methionine-independent revertants can be selected from populations of methionine-addicted cancer cells in culture, by treatment with recombinant methioninase, at various concentrations to restrict methionine in the medium, or by using methionine-free medium. The rare cells which survive methionine restriction and can continue to proliferate under methionine restriction, were found to be methionine-independent revertants, which have reduced malignancy (11, 15, 16). The methionine-independent revertant cells acquire a more-normal phenotype, including loss of ability to grow as clones in semi-solid medium (16, 17) and display reduced hyper-transmethylation (6), including greatly reduced methylation of histone H3 lysine marks (11).

The present report describes the loss of tumorigenic potential of the highly-malignant human lung-cancer cell line H460 when it reverts from methionine addiction to methionine independence.

Materials and Methods

Cell culture. Human lung-cancer cell line H460 used in this study is from the NCI-60 cell-line collection (18). We have shown that

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Key Words: Cancer, methionine addiction, reversion, methionine independence, tumorigenicity, lung cancer, H460.

H460 has very-high metastatic potential when transplanted orthotopically (19). Methionine-independent revertant H460-R1 cells were isolated from parental methionine-addicted H460 cancer cells, as previously described in medium containing recombinant methioninase to effect methionine restriction (11).

Animal studies. Athymic nu/nu mice (AntiCancer Inc, San Diego, CA, USA), 4-6 weeks old, were used in this study. All mice were kept in a barrier facility on a high-efficacy particulate air (HEPA)-filtered rack under standard conditions of 12 h light/dark cycles. Animal studies were performed with an AntiCancer Institutional Animal Care and Use Committee (IACUC)-protocol specially approved for this study, and in accordance with the principles and procedures outlined in the National Institutes of Health Guide for the Care and Use of Animals under Assurance Number A3873-1.

Cancer-cell transplantation to mice. Seven different doses of methionine-addicted H460 parental cancer cells or methionine-independent H460-R1 revertant cells (2×10^6, 1×10^6, 5×10^5, 2.5×10^5, 1×10^5, 5×10^4 or 1×10^4 cells/100 μl PBS), were injected subcutaneously into the flanks of nude mice. Each group comprised five mice.

Results

When as few as 5×10^4 methionine-addicted parental H460 cells were transplanted to nude mice they formed tumors. The methionine-independent H460-R1 revertant cells did not form tumors, even when as many as 2.5×10^5 cells were injected to nude mice (Figure 1). Reversion to methionine independence resulted in a large loss of tumorigenic potential of H460.

Discussion

The results of the present study further demonstrate the linkage of methionine addiction and malignancy (6, 15-17). We have previously reported that the H460-R1 revertant cells lost overmethylation of histone H3 lysine marks, which is associated with malignancy (11). Methionine addiction appears to be a universal hallmark of cancer (5, 20-22) and its study has revealed fundamental molecular changes required for oncogenic transformation (1-6, 10, 11). The importance of methionine addiction of cancer is becoming widely recognized (9, 23). Revertants such as the H460-R1 cells will be used to further select even more-normal revertants that can be used to understand the critical role of histone H3 lysine methylation in cancer. Methionine addiction has also been shown to be an important therapeutic target using methionine restriction by diet (8, 9, 13, 24, 25, 26) and by recombinant methioninase, which is especially promising due to the efficacy of orally-administered recombinant methioninase, observed in patient-derived orthotopic xenograft (PDOX) mouse models (22) and cancer patients (27).

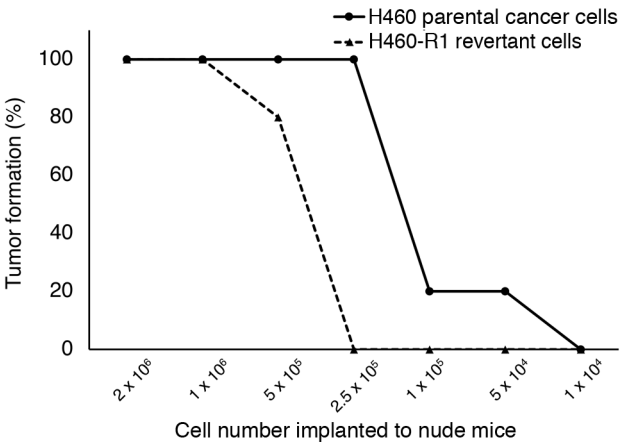

Figure 1. *Frequency of tumor formation in nude mice by methionine-addicted parental H460 cancer cells and methionine-independent revertant H460-R1 cells, implanted with different cell numbers.*

Conflicts of Interest

JY, NS, YS, SI, KH, HN, YA, SI and RMH are or were unsalaried associates of AntiCancer Inc. QH is an employee of AntiCancer Inc. The Authors declare that there are no potential conflicts of interest regarding this study.

Authors' Contributions

JY. and RMH designed and performed experiments and wrote the paper; QH, NS, YS, KH, YA, HN SI, KM, RM and MB gave technical support and conceptual advice. Writing, review, and/or revision of the manuscript: JY, IE and RMH.

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