

■ MOLECULAR BIOLOGY

Thioredoxin Reductase 1: A Double-edged Sword in Cancer Prevention and Promotion

Yoo MH, Xu XM, Carlson BA, Gladyshev VN, and Hatfield DL. Thioredoxin reductase 1 deficiency reverses tumor phenotype and tumorigenicity of lung carcinoma cells. *J Biol Chem* 281: 13005–8, 2006.

Dietary selenium has potent cancer prevention activity. Both low-molecular-weight selenocompounds and selenoproteins have been implicated in this activity. The major effect of selenium in health, however, is likely through the action of selenoproteins (Hatfield DL, Berry MJ, Gladyshev VN [Eds]. *Selenium: Its Molecular Biology and Role in Human Health*. Springer: New York, NY, 2006). Thioredoxin reductase 1 (TR1) is one of 25 known selenoproteins in humans and is a major antioxidant and redox regulator in mammalian cells. Interestingly, this enzyme appears to have opposing effects in cancer development, as it has been implicated in both cancer prevention and cancer promotion. For example, TR1 supports p53 function and has other tumor suppressor activities, and its inhibition by carcinogenic, electrophilic compounds further suggests a role in cancer prevention. On the other hand, TR1 is overexpressed in many cancer cells, and its inhibition by a variety of potent agents has been shown to alter the cancer-related properties of numerous tumors and malignant cells, leading several investigators to propose this enzyme as a possible target for cancer therapy.

It is not clear whether the cancer-preventing or cancer-promoting properties of TR1 influence cancer development more. To help determine this, we directly examined the role of this enzyme in a cancer cell line and in a mouse model.

We used RNA interference to specifically target and knockdown TR1 activity. Mouse Lewis lung carcinoma (LLC1) cells were stably transfected with the target vector and a control vector that had the same DNA sequence but lacked the targeting sequence. Two separate sites within the 3'-untranslated region of *TR1* were initially targeted because they were found to have very similar effects on reducing TR1 expression, which ruled out any possibility of off-targeting. The level of TR1 was substantially reduced in both these knockdown cell lines compared with the control cell line, as determined by Northern and Western blot analyses, 75-selenium labeling that specifically labeled the selenocysteine residue in TR1 (and other selenoproteins), and by direct assay of enzyme activity.

LLC1 cells transfected with the TR1 target had a number of altered properties that were

more in line with normal cells than with the LLC1 cells transfected with the control vector. For example, the TR1-deficient cells manifested a retarded growth rate compared with control cells. Other characteristics of LLC1 cells were also altered: For example, the control cells grew to be multilayered and loosely attached to the culture dishes. They also grew non-anchored in soft agar. In contrast, the TR1-inhibited cells grew in monolayers and were tightly attached to culture dishes. Also, their growth in soft agar was inhibited. Moreover, the expression of at least two cancer-related mRNAs, those of hepatocyte growth factor and osteopontin, was substantially reduced in TR1-inhibited cells.

Most significantly, mice injected with LLC1 cells that carried the TR1-targeting vector manifested a dramatic reduction in tumor progression and metastasis compared with mice injected with cells carrying the control vector. Tumorigenesis was examined by injecting three mice in the flank with TR1 knockdown cells and three mice with control cells. After two weeks, the mice were euthanized, the tumors excised and weighed, and the weights averaged. Tumors were much larger in mice injected with control cells, with an average weight of 0.341 g compared with an average weight of 0.063 g in mice injected with the TR1 knockdown cells. Moreover, the smaller tumors that arose from the TR1-deficient cells had lost the targeting vector, suggesting that TR1 is essential for tumor growth. Tumor metastasis was analyzed by injecting tail veins from mice with TR1 knockdown cells and control cells. The mice that received the injections were euthanized after 4 weeks, and their lungs were removed. Lungs from mice injected with the control cells had extensive tumor formation, whereas the lungs from mice injected with the TR1 knockdown cells had no visible tumors. Pathological analysis of lung slices showed widespread malignancy in mice injected with control cells, but only normal tissue in mice injected with the TR1 knockdown cells.

Overall, our study demonstrated that downregulating TR1 expression reverts the phenotype of malignant cells, making it more in line with that of normal cells. These observations provide direct evidence that the reduction of TR1 levels in malignant cells is antitumorigenic.

How can this apparently essential function of TR1 in cancer development be reconciled with the role of this enzyme in tumor suppression as well as the known anti-cancer role of selenium, which is a catalytic component of TR1? We propose that an adequate amount of dietary selenium in general, and a normal expression level of TR1 in particular, maintain cellular redox homeostasis in normal cells, protecting them against oxidative stress, DNA mutations, and damage to other cellular components. Each of these roles of selenium and TR1 are functions in which both components have been implicated. However, in newly emerging tumors, TR1 would be required to sustain tumor growth, likely because of the increased demand for its reducing equivalents. All of this would explain both the potent cancer prevention activity of dietary selenium and the role of TR1 as a double-edged sword in preventing and promoting cancer. Furthermore, our study provides the basis to explain disparate data in the literature on the role of this enigmatic protein in cancer and elevates TR1 to a prime target for cancer therapy.

Such studies as the present one are having a major impact on how we envision the dietary intake of selenium in humans and other mammals. It has been known for some time that diets containing sufficient or supplemental amounts of selenium have beneficial effects in preventing certain forms of cancer, possibly through the action of enriching the selenoprotein population. However, once cancer is initiated, then adequate or enriched amounts of selenium in the diet might serve to drive tumorigenesis.

Dolph Hatfield, PhD

Senior Investigator

Laboratory of Cancer Prevention

NCI-Bethesda, Bldg. 37/Rm. 6032A

Tel: 301-496-2797

Fax: 301-435-4957

hatfield@mail.nih.gov
