Human Manganese Superoxide Dismutase Target Sequence Polymorphism and Ovarian Cancer

Sir,

A low level of reactive oxygen species (ROS) generated during the normal metabolism is indispensible for several cellular processes.[1] However, excessive presence of ROS causes cellular stress which may lead to different pathological conditions including cancer, mainly by damaging deoxyribonucleic acid (DNA) or by altering key cellular processes.

Antioxidant enzymes play a significant role in maintaining normal ROS level in the cell. Thus, their altered expression may result in harmful consequences. Manganese superoxide dismutase (MnSOD) is a primary antioxidant enzyme that neutralizes highly reactive mitochondrial superoxide radical (O_2^-) to hydrogen peroxide (H_2O_2), which may be further neutralized by subsequent enzymes. Thus, MnSOD constitutes first line of defense in the cell against oxidative stress. A single nucleotide polymorphism from *T* to *C* at nucleotide 47 of its target sequence results in a valine (*GTT*) to alanine (*GCT*) change at codon 16. This val[16]ala polymorphism leads to a β-sheet conformation instead of a preferred α-helical structure of MnSOD precursor protein causing an impaired transport of MnSOD to mitochondria,[2] which would influence cellular ROS level.

Ovarian cancer is the fifth most frequent cancer in women.[3] Ovarian cell-damage caused due to excess production of ROS during ovulation can be a strong etiological factor for this cancer.[3] Therefore, genetic variation of MnSOD may influence the development of ovarian cancer. Here we report distribution of alleles for MnSOD val[16]ala polymorphism in ovarian cancer patients.

This was not a case-control study. It was carried out within a study designed to evaluate genetic predisposition for platinum-drug related toxicity in cancer patients supported by State Biotechnology Department, Uttarakhand. Cancer patients taking treatment with platinum drugs at Swami Ram Cancer Hospital and Research Center, Haldwani were recruited as study subjects for the original study. Among them 20 patients including 8 ovarian cancer patients were studied for MnSOD val[16]ala polymorphism. Signed informed consent was collected from each study participant. Ethical approval for the study was obtained from Institutional Ethical Committee, Government Medical College, Haldwani.

A total volume of 2-5 ml of peripheral blood was collected in sterile ethylenediaminetetraacetic acid tube. DNA was extracted from whole blood following standard phenol-chloroform method.[4] Polymerase chain reaction (PCR) was carried out using forward and reverse primers, 5′ AGCACCAGCAGGCAGCTGGCTCCG 3′ and 5′ CGGTGACGTTCAGGTTGTTCACG 3′, respectively (Eurofins, Bangalore). PCR was performed in a 25 μl of reaction volume using 10 pmol of each primer, dNTP mix (200 μM of each dNTP), 0.1 U of Taq polymerase (Promega, USA) and PCR buffer provided along with the enzyme. PCR condition followed an initial denaturation at 94°C for 4 min followed by 35 cycles of 30 s denaturation at 94°C, 30 s annealing at 63°C, 30 s extension at 72°C and final extension at 72°C for 5 min. Each set of reaction had negative control. PCR products were sequenced (SciGenome, India) thereafter.

It was observed that C allele and T allele were almost equally distributed in the study group (C and T allele frequency 0.48 and 0.52, respectively). However, C allele was more concentrated in the ovarian cancer patients (C allele frequency 0.75). Although frequency of MnSOD genotypes in normal individuals is not known for this population, literature[5] shows that *Ala* morph is maintained in a low frequency in healthy Asians (0.14-0.21). Assuming similar frequency for the present population, it can be said that *Ala* morph had a higher frequency in cancer patients (0.48) and it was even higher in ovarian cancer patients. Thus *Ala* morph may be linked to the development of ovarian cancer. However, this is a preliminary observation, which can be confirmed in a proper case-control study only.

A study by Sutton *et al*.[6] demonstrated that *Ala* morph of MnSOD was 30-40% more efficiently localized to the mitochondria than the *Val* morph. Thus there would be more conversion of O_2^- to H_2O_2 in comparison to *Val* morph. H_2O_2, if not neutralized, can increase mutation as well as metastasis and decreases apoptosis all of which may contribute towards cancer.[7] Thus *Ala* morph may be associated with cancer risk. It has been found to be associated with breast cancer, prostate cancer and other cancer types,[7] but rarely for ovarian cancer. Considering the facts that ROS induces ovarian cancer[3] and MnSOD is over expressed in ovarian cancer tissues,[8] further experiments on this polymorphism would help in better understanding of ovarian carcinogenesis.

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