Volume-4, Issue-7, July-2015 • ISSN No 2277 - 8160

Research Paper Biotechnology

Implications of Free Radicals in Different Stages of Breast Cancer

Neha Sharma	Department of Biotechnology, Kamla Raja Girls P.G. (Auto.) College, Gwalior (M P) India
D.S. Rathore	Department of Biotechnology, Kamla Raja Girls P.G. (Auto.) College, Gwalior (M P) India
Meenu Rai	Principal, College of life sciences, Cancer Hospital and Research Institute, Gwalior (M P) India

ABSTRACT

Researchers have recently shown an increased interest in free radicals and their role in the tumor microenvironment. Free radicals are molecules with high instability and reactivity due to the presence of an odd number of electrons in the outermost orbit of their atoms. Free radicals include reactive oxygen and nitrogen species, which are key players in the initiation and progression of tumor cells and enhance their metastatic potential. In fact, they are now considered a hallmark of cancer. However, both reactive species may contribute to improve the outcomes of radiotherapy in cancer patients. Besides, high levels of reactive oxygen species may be indicators of genotoxic damage in non-irradiated normal tissues. The purpose of this article is to review recent research on free radicals and carcinogenesis in order to understand the pathways that contribute to tumor malignancy. This work involves the free radicals in relevant cellular events, including their effects on DNA damage. This knowledge is crucial for evaluating the relevance of free radicals as therapeutic targets in cancer.

KEYWORDS : free radicals, carcinogenesis, DNA damage.

Introduction

The role of free radicals in the genesis of different diseases has been widely documented (Okezie *et al*. 1991 Polidori *et al*. 2001; Jomova & Valko 2011). Besides having specific cell functions, they can become toxic for the cells that produce them or for neighboring cells in contact in a tissue or organ. This is the case of oxygen, a highly stable molecule, which can turn into different reactive species, some with the character of free radicals, after participating in some cell metabolism functions. These free radicals constitute the product or are used to perform important cell functions, especially when the reactivity of molecular oxygen is insufficient (Turi *et al*. 2002).

The cell generates free radicals and also degrades that which is strictly necessary to avoid the damage derived from a non-controlled formation. However, various intrinsic and extrinsic circumstances and the biochemical activity of the cell can make it lose control over the formation and management of free radicals. This imbalance in the formation and use of free radicals in tissue is known as "oxidative stress". It results from a disturbance of the balance between the formation of reactive oxygen species (ROS) and the defense provided by cell antioxidants (Schafer & Buettner 2001; Shen *et al*. 2011). The application of chemotherapy in cancer treatments can also favor oxidative stress (Halliwell & Gutteridge 2007; Panis *et al*. 2012).

ROS and RNS contribute in different ways to carcinogenesis and the malignant progression of tumor cells, enhancing their metastatic potential. In fact, they are now considered a distinctive characteristic of cancer. These species lead to genomic damage and genetic instability, and they participate as intermediaries in mitogenic and survival signals *via* growth factor receptors and adhesion molecules, promoting cell mobility, inducing inflammation/repair and angiogenesis in the tumor microenvironment (Pani *et al*. 2010; Klaunig *et al*. 2010; Pande *et al*. 2011; Fuchs-Tarlovsky 2013; Pervin *et al*. 2013). Free radicals may produce breaks and considerable damage in the DNA molecule, producing mutations and eventually cancer. The main source of mutations in live organisms is DNA damage by oxidation, with an estimated frequency of 10^4 lesions/cell/day in human cells (Klaunig *et al*. 2010).

DNA is major target of free radical damage The types of damages include strand breaks (single or double strand breaks), various forms of base damage yielding products such as 8-hydroxyguanosine, thymine glycol or abasic sites, damage to deoxyribose sugar as well as DNA protein cross links. These damages can result in mutations that are heritable change in the DNA that can yield cancer in somatic cells or fetal malformations in the germ cells. The involvement of free radicals with tumor suppressor genes and proto-oncogenes suggest their role in the development of different human cancers (Halliwell *at el,* 1993). Oxidative damage to DNA is a result of interaction of DNA with ROS or RNS. Free radicals such as ·OH, and H· react with DNA by addition to bases or abstractions of hydrogen atoms from the sugar moiety. The C4-C5 double bond of pyrimidine is particularly sensitive to attack by ·OH, generating a spectrum of oxidative pyrimidine damage products, including thymine glycol, uracil glycol, urea residue, 5-hydroxydeoxyuridine, 5-hydroxydeoxycytidine and others. Similarly, interaction of ·OH with purines will generate 8-hydroxydeoxyguanosine (8-OHdG), 8-hydroxy deoxyadenosine, formamidopyrimidines and other less characterized purine oxidative products. Several repair pathways repair DNA damage. 8-OHdG has been implicated in carcinogenesis and is considered a reliable marker for oxidative DNA damage. Nucleic acids are pentose-phosphate polymers that can undergo reactions with hydroxyl radical like those depicted for hyaluronic acid. In addition there are several important examples of modifications to the base portion of the polymer. In fact these base modifications may be responsible for genetic defects produced by oxidative stress. Recently, 8-hydroxy guanosine has generated considerable interest as a product of hydroxyl radical attack on DNA that can be used to estimate DNA damage in humans (Halliwell and Aruoma, 1993).

Materials and methods

Histopathologically positive proved cases were selected for the present study. 97 healthy subjects attending camps organized by Cancer Hospital & Research Institute and 157 confirmed cases coming from the Cancer Hospital for treatment were considered for above study. The blood samples of above control and patients were collected for DNA damaged.

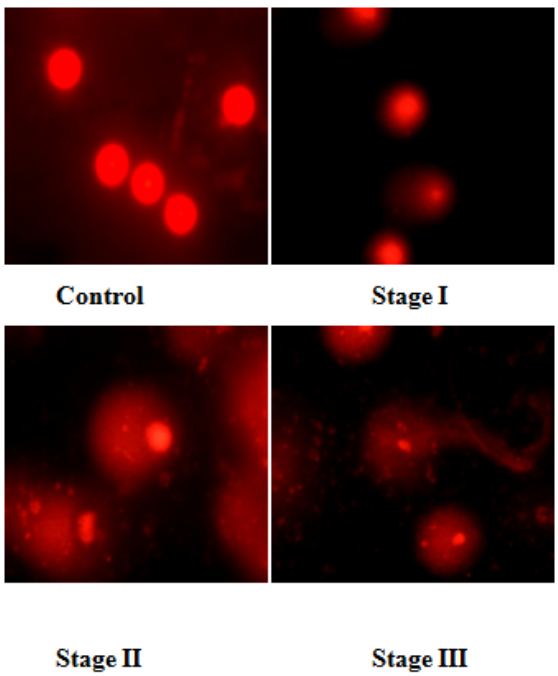
The subjects were divided into following groups on the basis of disorder:

- Normal healthy subjects
- Stage I subjects
- Stage II subjects
- Stage III subjects

DNA damage was assayed by the method of Sasaki *et al* (1997) with minor modification

Result

Study of DNA damage in lymphocytes of cancer patients showed significant damage. DNA damage in breast cancer patients was studied by comet assay. Quantization of DNA damage can be done by measurement of comet tail length. The result pointed out that comet tail was significantly increased in different stages of breast cancer when compared with control. The comet tail length is larger in patients of stage third cancer, which showed excessive DNA damage in breast cancer patients.



Discussion

Free radicals are highly unstable and reactive due to the presence of an odd number of electrons in the outermost orbit of their atoms; their aggressive action derives from their attempts to attain "balance" by binding with electrons of neighboring atoms, giving rise to chain reactions (Halliwell1999; Griendling *et al*. 2000).

Various cellular metabolic systems constantly produce free radicals from oxygen. Thus, 80% of molecular oxygen is consumed in mitochondria, and 5% of this is transformed into superoxide and hydroxyl radicals. Endogenous (prostaglandins, fatty acids, etc.) and exogenous (drugs, colorants, flavorings, antioxidants, etc.) substances are metabolized in the smooth endoplasmic reticulum, consuming 15% of molecular oxygen, of which 20-30% is reported to be transformed into free radicals, especially ·OH. Macrophages and leucocytes, as defense mechanisms against bacteria and virus, also contribute to the formation of free radicals. Free radicals are used in prostaglandin synthesis, as in the synthesis of cholesterol and steroidal hormones. The hydroxylation of lysine and proline amino acids to hydroxylysine and hydroxyproline, respectively, necessary for collagen biosynthesis, requires the participation of hydroxyl free radicals (Wright *et al*. 1994).

Hence, free radicals have an essential function in the normal metabolism of cells. However, their presence poses a risk, especially for large molecules, e.g., nucleic acids, proteins, polymerized carbohydrates (polysaccharides), and lipids, which are preferentially damaged by oxygenated free radicals; (Wright *et al*. 1994; Ferrari *et al*. 2009; Ziech *et al*. 2010).

Free radicals (super oxide, hydroxyl radicals and nitric oxide) and other reactive species (hydrogen peroxide, hypochloric acid and proxy nitrite) are produced during aerobic metabolism in the body. Actually in living cells, mitochondria (oxidative phosphorylation), leukocytes (oxidative burst), peroxisomes (degradation of fatty acids) and cytochromep450 system may contribute to the production of reactive oxygen species (ROS) in normal metabolism ROS occurring in vivo can cause oxidative damage of amino acids, lipids, proteins and DNA. Apart from in vivo ROS, DNA can also be damaged through exogenous ROS sources including cigarette smoking, UV and ionizing radiation. Free radicals have two faces in the body. They play as stimulators of signal transduction (e.g. Ca2+ signaling and protein phosphorylation) and regulatory molecules at physiologic levels whereas; they are highly cytotoxic oxidants at pathologic levels. Excess ROS may induce the oxidative damage including DNA strand breaks, base modifications and chromosomal aberrations. Available evidence has shown that DNA damage can result from free radical attacks if not repaired, the damage may lead to deteriorated gene expression, development of a number of diseases such as cancer, diabetes, neurodegenerative and vascular diseases and also aging. Especially in development of atherosclerosis, ROS have important roles including promoting cell proliferation, hypertrophy, growth arrest, apoptosis and oxidation of LDL. (Derya Özsavc, 2007)

Oxidative mechanisms have been demonstrated to possess a potential role in the initiation, promotion, and malignant conversion (progression) stages of carcinogenesis. Given that cumulative cancer risk increases with the age and is associated with an accumulation of DNA damage, oxidative DNA damage has been investigated in cancer. Lesions such as 8-OH-dG are established biomarkers of oxidative stress; coupled with their potential mutagenicity in mammalian cells, this has led to their proposed potential as intermediate markers of a disease endpoint—for example, cancer. Numerous studies have attempted to establish a relationship between levels of oxidative DNA damage and cancer. Elevated levels of damage are reported to arise as a consequence of an environment where in antioxidant enzymes and high in ROS generation. It has been reported that at least some tumor cell lines can produce significant levels of H_2O_2, without exogenous stimulation, perhaps accounting for the elevated levels of oxidative DNA damage seen. As a result of elevated ROS, transcription factors and their corresponding genes are permanently activated, which, coupled with increased DNA damage, creates a selection pressure for a malignant phenotype seen in cancer. Although such studies have furthered the hypothesis that oxidative DNA damage may be an important risk factor for carcinogenesis, it has been argued that the mere presence of 8-OH-dG in DNA is unlikely to be necessary or sufficient to cause tumor formation. (Marcus *et al,* 2003).

In our present study lymphocytes of cancer patients showed significant DNA damage. DNA damage in breast cancer patients was studied by comet assay. The result pointed out that comet tail was significantly increased in different stages of breast cancer when compared with control. The comet tail length is larger in patients of stage third cancer, which showed excessive DNA damage in breast cancer patients. Previous studies have suggested that elevated DNA damage levels may be associated with breast cancer risk. (Djuric, *et al* 2001; Hu, *et al* 2002).

REFERENCES

1. Derya Özsavc (2007). Oxidative DNA damage and repair system. Adv Mol Med; 3(2): 57-61 | 2. Djuric Z., Heilbrun LK., Lababidi S., Berzinkas E., Simon MS. and Kosir MA. (2001). Levels of 5-hydroxymethyl-2'-deoxyuridine in DNA from blood of women scheduled for breast biopsy. Cancer Epidemiol. Biomarkers Prev., 10, 147–149 | 3. Ferrari CKB, Franca EL, Honorio-Franca AC (2009) Nitric oxide, health and disease. J Appl Biomed 7:163-173 | 4. Fuchs-Tarlovsky V (2013) Role of antioxidants in cancer therapy. Nutrition (Burbank, Los Angeles County, Calif) 29(1):15-21. | 5. Griendling KK, Sorescu D, LassÃ¨gue B, Ushio-Fukai M (2000) Modulation of protein kinase activity and gene expression by reactive oxygen species and their role in vascular physiology and pathophysiology. Arteriosclerosis, thrombosis, and vascular biology 20(10):2175-2183. | 6. Halliwell B, Gutteridge J (2007) Free radicals in biology and medicine. New York: Oxford University. | 7. Halliwell B. and Aruoma OI. (1993). DNA and Free Radicals, Boca Raton Press. | 8. Hu JJ., Mohrenweiser HW., Bell DA., Leadon SA. and Miller MS. (2002). Symposium overview: genetic polymorphisms in DNA repair and cancer risk. Toxicol. Appl. Pharmacol., 185, 64–73. | 9. Jomova K, Valko M (2011) Importance of iron chelation in free radical-induced oxidative stress and human disease. Curr Pharm Des 17(31):3460-3473 | 10. Klaunig JE, Kamendulis LM, Hocevar BA (2010) Oxidative stress and oxidative damage in carcinogenesis. Toxicologic pathology 38(1):96-109 | 11. Marcus S. Cooke., Mark D. Evans., Miral Dizdaroglu and Joseph Lunec. (2003) Oxidative DNA damage: mechanisms, mutation, and disease The FASEB Journal; 17:1195-1214. | 12. Okezie I, Harparkash K, Haliwell B (1991) Oxygen free radicals and human diseases. J Roy Soc Health 34:171-177. | 13. Pande D, Negi R, Khanna S, Khanna R, Khanna HD (2011) Vascular endothelial growth factor levels in relation to oxidative damage and antioxidant status in patients with breast cancer. Journal of breast cancer 14(3):181-184. | 14. Pani G, Galeotti T, Chiarugi P (2010) Metastasis: cancer cell's escape from oxidative stress. Cancer Metastasis Rev 29(2):351-378 . | 15. Panis C, Herrera AC, Victorino VJ, Campos FC, Freitas LF, De Rossi T, Colado Simão AN, Cecchini AL, Cecchini R (2012) Oxidative stress and hematological profiles of advanced breast cancerpatients subjected to paclitaxel or doxorubicin chemotherapy. Breast Cancer Res Treat 133(1):89-97. | 16. Pervin S, Tran L, Urman R, Braga M, Parveen M, Li S, Chaudhuri G, Singh R (2013) Oxidative stress specifically downregulates survivin to promote breast tumour formation. Brit J Cancer 108(4):848-858. | 17. Polidori MC, Stahl W, Eichler O, Niestroj I, Sies H (2001) Profiles of antioxidants in human plasma. Free Radical Biol Med 30(5):456-462. | 18. Sasaki YF., Izumiyama F., Nishidate E., Matsusaka N. and Tsuda (1997): Detection of rodent liver carcinogen genotoxicity by the alkaline sigle gel electrophoresis (comet assay) in multiple mouse organ (Liver, spleen, lung, kidney, and bone marrow) Mut at Res. 39, 201-214 | 19. Schafer FQ, Buettner GR (2001) Redox environment of the cell as viewed through the redox state of the glutathione disulfide/glutathione couple. Free Radical Biol Med 30(11):1191-1212. | 20. Shen K, Ji L, Chen Y, Yu Q, Wang Z (2011) Influence of glutathione levels and activity of glutathione-related enzymes in the brains of tumor-bearing mice. Bioscience trends 5(1):30-37 | 21. Turi JL, Jaspers I, Dailey LA, Madden MC, Brighton LE, Carter JD, Nozik-Grayck E, Piantadosi CA, Ghio AJ (2002) Oxidative stress activates anion exchange protein 2 and AP-1 in airway epithelial cells. Am J Physiol Lung Cell Mol Physiol 283(4):L791-L798 | 22. Wright DT, Cohn LA, Li H, Fischer B, Li CM, Adler KB (1994) Interactions of oxygen radicals with airway epithelium. Environmental health perspectives 102(Suppl 10):85 . | 23. Ziech D, Franco R, Georgakilas AG, Georgakila S, Malamou-Mitsi V, Schoneveld O, Pappa A, Panayiotidis MI (2010) The role of reactive oxygen species and oxidative stress in environmental carcinogenesis and biomarker development. Chemico-biological interactions 188(2):334-339.