COMMENTARY

Endocrine-Related Cancer (2012) **19** C7–C11

Oxidative stress: a new risk factor for thyroid cancer

Mingzhao Xing

Laboratory for Cellular and Molecular Thyroid Research, Division of Endocrinology and Metabolism, Johns Hopkins University School of Medicine, Baltimore, Maryland 21287, USA

(Correspondence should be addressed to M Xing; Email: mxing1@jhmi.edu)

Abstract

Oxidative stress (OS) is a state of excessive free radicals and reactive metabolites among which the most important class is reactive oxygen species (ROS) – radicals derived from oxygen – as represented by the superoxide anion radical ($O_2^{\bullet-}$) and its reactive metabolites, hydroxyl radical ($^{\bullet}OH$) and hydrogen peroxide (H_2O_2). In essence, OS represents an imbalance between the production of oxidants – ROS – and their elimination by antioxidative systems in the body. Many studies have linked OS to thyroid cancer by showing its association with abnormally regulated oxidative or antioxidative molecules. The study by Wang *et al.* in the December 2011 issue of *Endocrine-Related Cancer* (**18**, 773–782) further supports this relationship by demonstrating a high total oxidant status and OS index in thyroid cancer patients. The origin of ROS in thyroid cancer patients has not been defined, but thyroid cancer itself can be one since inflammation, a major event in it, is a classical source of ROS. ROS may in turn enhance the mitogen-activated protein (MAP) kinase and phosphatidylinositol-3-kinase (PI3K) pathways, forming a vicious cycle propelling thyroid tumorigenesis. Regardless of the mechanism, the clinical implication of the association of OS with thyroid cancer is severalfold: one, OS is a new risk factor for thyroid cancer; two, OS confers thyroid cancer patients an increased risk for cardiovascular diseases, degenerative neurological disorders, and other cancers that are classically associated with OS; and three, interference with OS may reduce this risk and be therapeutically beneficial to thyroid cancer itself in thyroid cancer patients. These interesting possibilities deserve further studies.

Endocrine-Related Cancer (2012) **19** C7–C11

Introduction

In recent years, many studies have linked oxidative stress (OS) to thyroid cancer (e.g. Senthil & Manoharan 2004, Akinci *et al.* 2008, Lassoued *et al.* 2010). These studies investigated the relationship of various individual oxidative or antioxidative molecules with thyroid cancer and provided evidence suggesting an association of OS with this cancer. In the study reported by Wang *et al.* (2011) in the December 2011 issue of *Endocrine-Related Cancer*, the authors took a further step to look at this issue and provided additional strong evidence to support an association of OS with thyroid cancer. A particularly important aspect of this study was the examination of total oxidant status and also OS index and demonstration of their strong association with thyroid cancer. These parameters likely fully reflect OS status in the body of a patient, and thus could more accurately reflect the significance of OS in relation to thyroid cancer. Moreover, a unique strength of the Wang *et al.* study was also the inclusion of a group of patients with autoimmune thyroid diseases for comparison. Patients with autoimmune thyroid diseases have been shown to be associated with an increased OS (e.g. Andryskowski & Owczarek 2007, Erdamar *et al.* 2008, Torun *et al.* 2009, Aslan *et al.* 2011). Interestingly, the Wang *et al.* study found a much stronger association of OS with thyroid cancer in comparison with autoimmune thyroid diseases, consistent with previous similar studies on individual oxidative or antioxidative molecules (Lassoued *et al.* 2010), suggesting a more important role of OS in thyroid cancer. Although the mechanism for the link between OS and thyroid cancer and whether there is a causal relationship between the two have not been directly investigated, the report of Wang *et al.*, together

1351–0088/12/019–C7
DOI: 10.1530/ERC-11-0360
Online version via http://www.endocrinology-journals.org

with the previous studies, seems to have now firmly established the association of the two.

Oxidative stress (OS) and reactive oxygen species (ROS)

The concept of OS has developed and evolved from the original free radical theory of oxygen toxicity nearly three scores of years ago (Commoner *et al*. 1954, Gerschman *et al*. 1954). OS represents a biochemical state of excessive presence of free radicals and reactive metabolites that exhibit harmful biological effects potentially damaging the organism (Valko *et al*. 2007, Durackova 2010). Free radicals are molecules or molecular fragments containing one or more unpaired electrons in atomic or molecular orbitals that confer the molecules considerable reactivity. Reactive oxygen species (ROS) – radicals derived from oxygen – are the most important class of radical species generated in organisms. Thus, OS represents an imbalance between the production of oxidants – ROS – and their elimination by the protective antioxidative systems in the body. A well-known basic radical ROS molecule is superoxide anion radical ($O_2^{\bullet -}$). Its metabolites, such as hydroxyl radical ($^{\bullet}OH$) and hydrogen peroxide (H_2O_2), are even more reactive. Another prominent radical system includes nitric oxide ($NO^{\bullet}$) and its metabolite peroxynitrite. ROS are products of normal cellular metabolisms. At low or moderate concentrations, they actually play an important beneficial role in certain physiological processes, such as cellular responses in defense against infectious agents and certain normal cellular signaling activities. Their excessive accumulation can cause damage of biological molecules and systems. The normal and important balance between beneficial and harmful effects of ROS in the body is maintained by redox regulation mechanisms that protect the body from OS (Dröge 2002). Not surprisingly, in humans, OS has been well known to be associated with major diseases, such as diabetes mellitus, atherosclerosis, hypertension, inflammatory diseases, neurodegenerative disorders, and other diseases (Valko *et al*. 2007, Durackova 2010).

Possible mechanisms of OS and ROS in thyroid tumorigenesis

As with thyroid cancer, OS has also been known to be associated with other cancers (Lu *et al*. 2006, Valko *et al*. 2007, Durackova 2010). Although it is not clear mechanistically how OS can play a role in the pathogenesis of thyroid cancer, it seems that excessive ROS as a consequence of imbalanced intracellular redox systems may be an important event in the molecular pathogenesis of thyroid cancer, as implicated by cancers induced by certain chemicals, such as asbestos-induced lung cancer (Stayner *et al*. 1996) and iron-induced colorectal cancer (Valko *et al*. 2001). One potential consequence of this event may be DNA damage by ROS, resulting in mutagenic genetic alterations that can initiate carcinogenesis and development of cancer. Perhaps such genetic alterations could include those that can constitutively activate major signaling pathways, such as the MAP kinase and PI3K/Akt pathways. In fact, previous studies have demonstrated that ROS, particularly H_2O_2, can activate the MAP kinase pathway signaling and consequent cellular proliferation (Rao & Berk 1992, Guyton *et al*. 1996, Aikawa *et al*. 1997). There are also studies showing that the PI3K/Akt and nuclear factor κB (NFκB) pathways can be activated by ROS (Poli *et al*. 2004, Spencer 2005, Durackova 2010). Thus, these studies demonstrate a direct role of ROS in affecting the major intracellular signaling pathways that are widely involved in human tumorigenesis.

Over activation of the MAP kinase and PI3K/Akt pathways is a fundamental mechanism in the tumorigenesis of thyroid cancer (Xing 2007, 2010). NFκB pathway also plays a role in thyroid tumorigenesis (Liu & Xing 2008, Pacifico & Leonardi 2010). It is thus plausible to propose that ROS may play a role in the pathogenesis of thyroid cancer through affecting these signaling pathways. Consequently, this may represent a mechanistic link of OS with thyroid cancer and make ROS a novel contributing factor to the pathogenesis of thyroid cancer – an interesting hypothesis that is tempting to test. As genetic alterations of the MAP kinase and PI3K/Akt pathways, such as the mutations in the *BRAF, Ras, PIK3CA*, and *PTEN* genes, are a dominant driving force for the activation of these pathways (Xing 2007, 2010), it would be interesting to see how ROS could interact or cooperate with these genetic alterations in promoting the pathogenesis and progression of thyroid cancer. Perhaps a similar clinical approach as used in the Wang *et al*. study could be employed to see how OS levels might be specifically linked to the aggressiveness of thyroid cancer with respect to various genetic backgrounds.

ROS can stimulate the production of matrix metalloproteinase and various cytokines such as transforming growth factor β1 (TGFβ1; Poli *et al*. 2004, Valko *et al*. 2007, Durackova 2010). This may potentially be another mechanism for the role of ROS in the pathogenesis of thyroid cancer. Recent studies have established that alterations in extracellular matrix

microenvironments involving abnormal production of matrix metalloproteinases and inflammatory cytokines are a major molecular mechanism in *BRAF* mutant-promoted pathogenesis and progression of thyroid cancer (Nucera *et al*. 2011). In this process, cytokine TGFβ1 has been shown to play a particularly important role (Riesco-Eizaguirre *et al*. 2009, Knauf *et al*. 2011). Interestingly, not only expression of TGFβ1 is increased under the state of increased OS but also ROS can be downstream intermediates of TGFβ1 (Poli *et al*. 2004). On the other hand, one study showed that under *in vitro* conditions, certain ROS-enhancing agents could somehow paradoxically induce degradation of the *BRAF* mutant (Fukuyo *et al*. 2008). Thus, *BRAF* mutant, TGFβ1, and ROS may have a complex relationship and role in the pathogenesis of thyroid cancer.

Sources of OS and ROS in thyroid cancer

If the mechanism in the link of OS with thyroid cancer involves an active role of ROS in promoting the pathogenesis of thyroid cancer as discussed above, there needs to be a source that persistently supplies ROS in the condition of thyroid cancer. In other words, a fundamental question is how excessive ROS is produced in patients with thyroid cancer. One answer may lie in the fact that thyroid cancer, like many other cancers (Lu *et al*. 2006), is, in many ways, an inflammatory disease. It has become well known in recent years that thyroid cancer is often abundantly infiltrated with inflammatory cells and various important inflammatory cytokines are actively produced in thyroid cancer (Nucera *et al*. 2011). Some of these inflammatory cytokines have an established important role in thyroid tumorigenesis, such as TGFβ1, as discussed above (Riesco-Eizaguirre *et al*. 2009, Knauf *et al*. 2011), and thrombospondin-1 (Nucera *et al*. 2010). Thus, inflammatory process in thyroid cancer and the ensuing development of inflammatory microenvironments seem to be an integral part of the pathogenesis of thyroid cancer. *BRAF* mutation, which is the most common activating mutation in thyroid cancer and is associated with tumor progression and aggressiveness (Xing *et al*. 2005), could elicit strong inflammatory responses of this cancer (Nucera *et al*. 2011). This inflammatory environment of thyroid cancer is conceivably a significant source of ROS since inflammation is a well-established classical condition that produces high OS (Poli *et al*. 2004, Lu *et al*. 2006). It is also possible that the common oncogenic mutants in thyroid cancer, such as the *BRAF* mutant, could increase the production of ROS through a yet-to-be-defined non-inflammatory mechanism. Thus, an interesting mechanism could be that oncogene-promoted production of ROS in turn promotes the pathogenesis and progression of thyroid cancer by enhancing the activation of major signaling pathways initiated by oncogenes, forming a vicious cycle that propels the pathogenesis of thyroid cancer.

There may be other mechanisms by which ROS can be produced in thyroid cancer, causing a high OS state. In fact, the thyroid gland itself is a site where reactive radical molecular species are actively generated through the process of iodide metabolism and thyroid hormone synthesis. In this process, signaling of thyrotropin (TSH) acting on the TSH receptor (TSHR) on thyroid cells stimulates the synthesis of H_2O_2, which is the substrate of thyroperoxidase in thyroglobulin iodination and thyroid hormone synthesis (Corvilain *et al*. 1991). ROS is therefore generated actively in the process of TSH stimulation of thyroid cells. Differentiated thyroid cancer cells usually express functional TSHR. It is thus conceivable that such thyroid cancer cells, upon stimulation by TSH secreted by the pituitary gland, could actively produce H_2O_2, thus contributing to the high OS state in thyroid cancer patients.

Interestingly, several studies in recent years have demonstrated a strong association of high TSH levels with an increased malignancy risk of thyroid nodules (Boelaert *et al*. 2006, Haymart *et al*. 2009). The mechanism of this association has been unclear. Given the link of OS with thyroid cancer and the possible mechanism involving ROS in promoting thyroid tumorigenesis discussed above, stimulation of H_2O_2 production by TSH acting on TSHR may represent one explanation. Such a mechanism is well consistent with the previous demonstrations that ROS can activate the thyroid cancer-promoting MAP kinase, PI3K/Akt, and NFκB pathways, as discussed above.

Clinical implications of OS in thyroid cancer

Whether OS-generating conditions other than the source of thyroid cancer itself tend to exist in patients with thyroid cancer remains to be investigated. Regardless of the source, the high OS state in thyroid cancer patients may also have important non-thyroid cancer health implications in these patients. For example, since OS is associated with cardiovascular disease, diabetes mellitus, neurodegenerative disorders, and other cancers, it remains an important question whether thyroid cancer patients are predisposed to these diseases, and may therefore warrant

appropriate surveillance and special prevention for such diseases. Further biological and epidemiological studies are needed to address this important issue. Whether interference in the metabolism of ROS to reduce OS by targeting the potential mechanisms discussed above or by taking antioxidative measures can be preventative and therapeutic for such diseases in thyroid cancer patients and for thyroid cancer itself remains to be an interesting question to answer.

Regardless of the source of high OS in thyroid cancer, the strong association of OS with thyroid cancer demonstrated by Wang *et al*. (2011) and other investigators in recent years and the plausible molecular mechanisms discussed above strongly suggest that high OS is a risk factor associated with thyroid tumorigenesis and likely a risk factor for the progression of thyroid cancer as well. There are very few known thyroid cancer risk factors, among which the best established are radiation exposure and family history of thyroid cancer, which are currently clinically used in the risk assessment of thyroid nodules (Cooper *et al*. 2009). Although most of the mechanistic aspects or hypotheses discussed above regarding the role of OS in thyroid cancer remain to be directly tested, it seems to be convincing that high OS represents a new risk factor for thyroid cancer. It is thus tempting to propose that testing of OS, particularly in the form of total oxidant status and OS index as proposed by Wang *et al*. (2011), may be clinically useful in the risk assessment of thyroid nodules.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

Funding

M Xing was supported by the National Institutes of Health R0-1 grant R01CA134225.

References

Aikawa R, Komuro I, Yamazaki T, Zou Y, Kudoh S, Tanaka M, Shiojima I, Hiroi Y & Yazaki Y 1997 Oxidative stress activates extracellular signal-regulated kinases through Src and Ras in cultured cardiac myocytes of neonatal rats. *Journal of Clinical Investigation* **100** 1813–1821. (doi:10.1172/JCI119709)

Akinci M, Kosova F, Cetin B, Sepici A, Altan N, Aslan S & Cetin A 2008 Oxidant/antioxidant balance in patients with thyroid cancer. *Acta Cirúrgica Brasileira* **23** 551–554. (doi:10.1590/S0102-86502008000600013)

Andryskowski G & Owczarek T 2007 The evaluation of selected oxidative stress parameters in patients with hyperthyroidism. *Polish Archives of Internal Medicine* **117** 285–289.

Aslan M, Cosar N, Celik H, Aksoy N, Dulger AC, Begenik H, Soyoral YU, Kucukoglu ME & Selek S 2011 Evaluation of oxidative status in patients with hyperthyroidism. *Endocrine* **40** 285–289. (doi:10.1007/s12020-011-9472-3)

Boelaert K, Horacek J, Holder RL, Watkinson JC, Sheppard MC & Franklyn JA 2006 Serum thyrotropin concentration as a novel predictor of malignancy in thyroid nodules investigated by fine-needle aspiration. *Journal of Clinical Endocrinology and Metabolism* **91** 4295–4301. (doi:10.1210/jc.2006-0527)

Commoner B, Townsend J & Pake GE 1954 Free radicals in biological materials. *Nature* **174** 689–691. (doi:10.1038/174689a0)

Cooper DS, Doherty GM, Haugen BR, Kloos RT, Lee SL, Mandel SJ, Mazzaferri EL, McIver B, Pacini F, Schlumberger M, *et al.* 2009 Revised American Thyroid Association management guidelines for patients with thyroid nodules and differentiated thyroid cancer. *Thyroid* **11** 1167–1214.

Corvilain B, van Sande J, Laurent E & Dumont JE 1991 The H_2O_2-generating system modulates protein iodination and the activity of the pentose phosphate pathway in dog thyroid. *Endocrinology* **128** 779–785. (doi:10.1210/endo-128-2-779)

Dröge W 2002 Free radicals in the physiological control of cell function. *Physiological Reviews* **82** 47–95.

Durackova Z 2010 Some current insights into oxidative stress. *Physiological Research* **59** 459–469.

Erdamar H, Demirci H, Yaman H, Erbil MK, Yakar T, Sancak B, Elbeg S, Biberoğlu G & Yetkin I 2008 The effect of hypothyroidism, hyperthyroidism, and their treatment on parameters of oxidative stress and antioxidant status. *Clinical Chemistry and Laboratory Medicine* **46** 1004–1010. (doi:10.1515/CCLM.2008.183)

Fukuyo Y, Inoue M, Nakajima T, Higashikubo R, Horikoshi NT, Hunt C, Usheva A, Freeman ML & Horikoshi N 2008 Oxidative stress plays a critical role in inactivating mutant BRAF by geldanamycin derivatives. *Cancer Research* **68** 6324–6330. (doi:10.1158/0008-5472.CAN-07-6602)

Gerschman R, Gilbert DL, Nye SW, Dwyer P & Fenn WO 1954 Oxygen poisoning and X-irradiation – a mechanism in common. *Science* **119** 623–626. (doi:10.1126/science.119.3097.623)

Guyton KZ, Liu Y, Gorospe M, Xu Q & Holbrook NJ 1996 Activation of mitogen-activated protein kinase by H_2O_2. Role in cell survival following oxidant injury. *Journal of Biological Chemistry* **271** 4138–4142. (doi:10.1074/jbc.271.7.3604)

Haymart MR, Glinberg SL, Liu J, Sippel RS, Jaume JC & Chen H 2009 Higher serum TSH in thyroid cancer patients occurs independent of age and correlates with extrathyroidal extension. *Clinical Endocrinology* **71** 434–439. (doi:10.1111/j.1365-2265.2008.03489.x)

Knauf JA, Sartor MA, Medvedovic M, Lundsmith E, Ryder M, Salzano M, Nikiforov YE, Giordano TJ, Ghossein RA & Fagin JA 2011 Progression of BRAF-induced thyroid cancer is associated with epithelial–mesenchymal transition requiring concomitant MAP kinase and TGFβ signaling. *Oncogene* **30** 3153–3162. (doi:10.1038/onc.2011.44)

Lassoued S, Mseddi M, Mnif F, Abid M, Guermazi F, Masmoudi H, El Feki A & Attia H 2010 A comparative study of the oxidative profile in Graves' disease, Hashimoto's thyroiditis, and papillary thyroid cancer. *Biological Trace Element Research* **138** 107–115. (doi:10.1007/s12011-010-8625-1)

Liu D & Xing M 2008 Potent inhibition of thyroid cancer cells by the MEK inhibitor PD0325901 and its potentiation by suppression of the PI3K and NF-kappaB pathways. *Thyroid* **18** 853–864. (doi:10.1089/thy.2007.0357)

Lu H, Ouyang W & Huang C 2006 Inflammation, a key event in cancer development. *Molecular Cancer Research* **4** 221–233. (doi:10.1158/1541-7786.MCR-05-0261)

Nucera C, Porrello A, Antonello ZA, Mekel M, Nehs MA, Giordano TJ, Gerald D, Benjamin LE, Priolo C, Puxeddu E *et al.* 2010 B-Raf(V600E) and thrombospondin-1 promote thyroid cancer progression. *PNAS* **107** 10649–10654. (doi:10.1073/pnas.1004934107)

Nucera C, Lawler J & Parangi S 2011 BRAF(V600E) and microenvironment in thyroid cancer: a functional link to drive cancer progression. *Cancer Research* **71** 2417–2422. (doi:10.1158/0008-5472.CAN-10-3844)

Pacifico F & Leonardi A 2010 Role of NF-kappaB in thyroid cancer. *Molecular and Cellular Endocrinology* **321** 29–35. (doi:10.1016/j.mce.2009.10.010)

Poli G, Leonarduzzi G, Biasi F & Chiarpotto E 2004 Oxidative stress and cell signalling. *Current Medicinal Chemistry* **11** 1163–1182.

Rao GN & Berk BC 1992 Active oxygen species stimulate vascular smooth muscle cell growth and proto-oncogene expression. *Circulation Research* **70** 593–599.

Riesco-Eizaguirre G, Rodríguez I, De la Vieja A, Costamagna E, Carrasco N, Nistal M & Santisteban P 2009 The BRAFV600E oncogene induces transforming growth factor beta secretion leading to sodium iodide symporter repression and increased malignancy in thyroid cancer. *Cancer Research* **69** 8317–8325. (doi:10.1158/0008-5472.CAN-09-1248)

Senthil N & Manoharan S 2004 Lipid peroxidation and antioxidants status in patients with papillary thyroid carcinoma in India. *Asia Pacific Journal of Clinical Nutrition* **13** 391–395.

Spencer JPE 2005 Interactions of flavonoids and their metabolites with cell signaling cascades. In *Nutrigenomics*, pp 353–377. Eds G Rimbach, J Fuchs & L Packer. Boca Raton: Taylor & Francis Publishing.

Stayner LT, Dankovic DA & Lemen RA 1996 Occupational exposure to chrysotile asbestos and cancer risk: A review of the amphibole hypothesis. *American Journal of Public Health* **86** 179–186. (doi:10.2105/AJPH.86.2.179)

Torun AN, Kulaksizoglu S, Kulaksizoglu M, Pamuk BO, Isbilen E & Tutuncu NB 2009 Serum total antioxidant status and lipid peroxidation marker malondialdehyde levels in overt and subclinical hypothyroidism. *Clinical Endocrinology* **70** 469–474. (doi:10.1111/j.1365-2265.2008.03348.x)

Valko M, Morris H, Mazur M, Rapta P & Bilton RF 2001 Oxygen free radical generating mechanisms in the colon: do the semiquinones of vitamin K play a role in the etiology of colon cancer? *Biochimica et Biophysica Acta* **1527** 161–166. (doi:10.1016/S0304-4165(01)00163-5)

Valko M, Leibfritz D, Moncol J, Cronin MT, Mazur M & Telser J 2007 Free radicals and antioxidants in normal physiological functions and human disease. *International Journal of Biochemistry & Cell Biology* **39** 44–84. (doi:10.1016/j.biocel.2006.07.001)

Wang D, Feng J, Zeng P, Yang Y, Luo J & Yang Y 2011 Total oxidant/antioxidant status in sera of patients with thyroid cancers. *Endocrine-Related Cancer* **18** 773–782. (doi:10.1530/ERC-11-0230)

Xing M 2007 *BRAF* mutation in papillary thyroid cancer: pathogenic role, molecular bases, and clinical implications. *Endocrine Reviews* **28** 742–762. (doi:10.1210/er.2007-0007)

Xing M 2010 Genetic alterations in the phosphatidylinositol-3 kinase/Akt pathway in thyroid cancer. *Thyroid* **20** 697–706. (doi:10.1089/thy.2010.1646)

Xing M, Westra WH, Tufano RP, Cohen Y, Rosenbaum E, Rhoden KJ, Carson KA, Vasko V, Larin A, Tallini G *et al.* 2005 *BRAF* mutation predicts a poorer clinical prognosis for papillary thyroid cancer. *Journal of Clinical Endocrinology and Metabolism* **90** 6373–6379. (doi:10.1210/jc.2005-0987)

Received in final form 1 December 2011
Accepted 1 December 2011
Made available online as an Accepted Preprint
1 December 2011