Thioredoxin and Thioredoxin Reductase Expression in Thyroid Cancer Depends on Tumour Aggressiveness

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Abstract. *Thyroid cancer is the second most common malignancy following breast cancer in Arab females. Thioredoxin (TRX) is a small multi-functional redox protein with both intracellular and extracellular functions. The protein exists in either a reduced form (thioredoxin-SH2) or an oxidized form (thioredoxin-S2). TRX acts as an enhancement for growth factors and stimulates the growth of cancer cells. In this study of thyroid neoplasms, involving 121 female and 62 male patients, expression of TRX and TRX-R was studied using purified mouse anti-human TRX monoclonal antibody and anti-human TRX-R antiserum from rabbits, respectively. In order to delineate tumour cell growth, proliferating cell nuclear antigen (PCNA) polyclonal antibody was used. Compared to normal thyroid tissue, expression of TRX and TRX-R was increased in the cytoplasm and nuclei of thyroid cancer cells. Furthermore, TRX expression correlated with that of TRX-R. Of the 183 thyroid neoplasms investigated, overexpression of TRX-R was found in different types of neoplasms. The majority of carcinomas showed a correlation between strongly positive TRX and TRX-R expression and neoplastic cellular proliferation, as measured by PCNA. This indicates that increased TRX and TRX-R expression may be associated with tumourigenesis by acting as an autocrine growth stimulus. This study suggests that TRX immunoreactivity in thyroid tumours is a function of malignancy and cancer progression. In addition, secreted TRX can also act as an extracellular growth factor for both normal and tumour cells and enhance the sensitivity of the cells. Furthermore, this study emphasizes the potential benefits of anti-TRX/TRX-R agents in cancer therapeutics in the treatment of thyroid cancer.*

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Thyroid cancer is a common endocrine malignancy with an incidence of approximately 0.5-10 cases per 100,000 (1) and represents 0.5-1% of human cancers. According to WHO guidelines, it is classified as papillary, follicular, anaplastic or medullary carcinoma and 80-90% are highly differentiated tumours (follicular and papillary) with good prognosis and five year survival of 95%. The anaplastic tumours are undifferentiated with a poor outcome. Thyroid cancer differs from many other neoplasms in that incidence and type vary widely in various geographical areas and it has a wide spectrum of clinical behaviour and therapeutic responsiveness. Follicular thyroid carcinoma (FTC) and anaplastic thyroid carcinoma (ATC) are more frequent in endemic goiter regions than in goiter free areas (2). Although FTC contributes a lesser percentage, it is necessary to have a clear-cut differentiation between PTC and FTC and clinical management and prognosis depend much on the diagnostic reliability of histopathological examination of the suspicious thyroid lesion.

Thyroid cancer and the majority of benign thyroid diseases (BTD) (*e.g.* nodule/adenoma, non-endemic goitre, hypo- and hyperthyroidism, Graves disease, thyroiditis) occur 3-5 times more frequently among women. This female predominance, greatest during reproductive ages, is observed in all geographical areas and ethnic groups. Evidence from epidemiological and laboratory studies suggest that reproductive factors and patterns may influence, or contribute to, the risk of BTD and thyroid cancer in women. Most cancers found during pregnancy are differentiated and have an excellent prognosis. However, thyroid nodules which develop in these patients appear to have a greater risk of malignancy and should be evaluated aggressively.

Exposure to high-dose of ionising radiation, particularly in childhood and adolescence, and previous hyperplastic thyroid disease (*i.e.* nodule/adenoma, goitre) are two well established risk factors for thyroid cancer. Radiation induces fibrosis within the capsule and decreases thyroid function (3). From the Chernobyl nuclear accident, it is known that radiation exposure is associated with an increased incidence of circulating antithyroid antibodies, inducing hypofunction of the thyroid. A relation between hypothyroidism and circulating antithyroid antibodies (*e.g.* thyroid peroxidase and thyroglobulin antibodies) has been found and may precede the development of hypothyroidism (4). Thyroid hormone influences the cell cycle of both normal and neoplastic cells and has a permissive role in the growth of certain solid tumours (5). Thyroid hormone receptors (THR) are transcription factors and act in a ligand-inducible manner by binding to DNA response elements in the regulatory regions of target genes. Potentially, THR mutations can recast gene expression profile in a cell, resulting in a tendency to tumourigenesis (6).

The mammalian thioredoxins (TRX) are a family of redox proteins that undergo NADPH-dependent reduction by thioredoxin reductase (TRX-R) and in turn reduce oxidized cysteine groups of proteins. The two main thioredoxins are thioredoxin-1 (TRX-1), a cytosolic and nuclear form, and thioredoxin-2 (TRX-2), found only as mitochondrial form. Human TRX-1 is a small multifunctional redox protein with both intracellular and extracellular functions. The protein exists in a reduced form (thioredoxin-SH_2). TRX-1 is a 104 amino acid protein, predominantly found in the cytosol. TRX-2 is found in the mitochondria and is a 166 amino acid protein containing the two cysteine residues (7).

The mechanism of TRX action involves a transient, mixed disulphide intermediate and a fast thiol-disulphide exchange in a hydrophobic environment. Thioredoxin is able to act as a powerful protein disulphide oxireductase through the reversible oxidation of its active dithiol centre and through this action it is able to affect the disulphide-dependent structural changes in other molecules, causing the activation or inhibition of them. Thioredoxin performs many biological actions including the supply of reducing equivalents to thioredoxins peroxidases and ribonucleotide reductase, the regulation of transcription factor activity, and the regulation of enzyme activity by heterodimer formation. Thioredoxin stimulates cell growth and is an inhibitor of apoptosis and may play a role in a variety of human diseases, including cancer. An increased level of thioredoxin has been reported in several human tumours, where it is associated with aggressive tumour growth (8-17).

An investigation of TRX is not complete without consideration of the role of thioredoxin reductase, the enzyme able to reduce the active site of TRX. Mammalian thioredoxin reductases are homodimeric, flavin adenine dinucleotide containing proteins with a C-terminal selenocysteine residue. The cys residues of the conserved redox catalytic site of mammalian thioredoxin reductases -Cys-Val-Asn-Val-Gly-Cys-, undergo reversible oxidation reduction in much the same way as TRX. The selenocysteine is essential for the activity of mammalian thioredoxin reductases. Two full-length human thioredoxin reductases have been cloned, a 54.4 kDa thioredoxin reductase-1, which is predominantly cytosolic, and a 56.2 kDa thioredoxin reductase-2, which has a 33 amino acid *N*-terminal extension identified as a mitochondrial import sequence (18). Thioredoxin reductase is able to inhibit the release of cytochrome *c* from the mitochondria to the cytosol, preventing hydrogen peroxide accumulation in the cells, thus inhibiting the cell signal to apoptosis, since hydrogen peroxide accentuates the apoptotic process. From these findings one can conclude that thioredoxin reductase inhibits cytochrome *C* release and accelerates cancer development. Here we investigate the expression of thioredoxin and thioredoxin reductase system in neoplasms of the human thyroid gland.

Materials and Methods

Tumour procurement and preparation. Thyroid tumour tissues were obtained from the Mubarek Al-Kaber Hospital, Jabriya, Al-Amiri Hospital, Safat and Cancer Control Centre, Shuwaikh, Kuwait, Arabian Gulf. The age of the patients ranged from 16 to 70 years with medium age of 36 years. The following thyroid neoplasms, obtained from 121 female and 62 male patients, were investigated: benign colloid nodule (n=15), colloid goitre (n=14), multinodular goitre (n=21), papillary oncocytic neoplasm (n=10), follicular adenoma (n=16), follicular carcinoma (n=24), invasive follicular carcinoma (n=19), papillary carcinoma (n=48), medullary carcinoma (n=9) and anaplastic carcinoma (n=7). Tissues were fixed in phosphate-buffered 4% paraformaldehyde (pH 7.4) and processed for paraffin embedding. Sections of 5 μm thickness were stained with haematoxylin-eosin or immunostaining procedures. Tissue located adjacent to tumours was used as controls.

Classification of thyroid malignancy. Histological differentiation of various thyroid swellings and the identification of the characteristic features of the malignancy were performed by applying standard WHO histological classification for thyroid cancers. According to the WHO classification, malignant tumours of the thyroid are subdivided into thyroid-specific, which are unique to the thyroid (papillary, follicular and medullary carcinoma), and tumours commonly found in other organs, but which still have some particular features when they occur in the thyroid gland (*e.g.* lymphoma, some types of sarcoma). Thyroid-specific tumours are derived from follicular cells (papillary and follicular carcinoma), and from parafollicular, calcitonin-producing C-cells (medullary carcinoma). Among the thyroid malignancies, papillary carcinoma is the most frequent type of follicular-cell derived carcinoma. Subtypes of papillary carcinoma are papillary microsarcoma, encapsulated variant, follicular variant, diffuse sclerosing variant and oxyphilic cell type. The variants of follicular carcinoma are:

minimally invasive (encapsulated), widely invasive, oxyphilic (oncocytic cell type) cell type and clear cell variants. All cases with confirmed histology were staged according to the TNM system recommended by the International Union Against Cancer.

Thioredoxin and thioredoxin-reductase immunohistochemistry. Mouse monoclonal anti-human TRX antibody 2B1 and rabbit monoclonal anti-human TRX-R were prepared (10). The presence of thioredoxin and thioredoxin reductase was demonstrated by the streptavidin-biotin horseradish peroxidase complex (ABC) technique. Sections were deparaffinized and endogenous peroxidase was blocked by application of 3% hydrogen peroxide in Tris buffer for 5 min at room temperature (RT), followed by rinsing in Tris buffer. Non-specific protein binding was blocked by incubation with 20% normal goat serum in PBS containing 1% bovine serum albumin (BSA). Positive controls were prepared using paraffin wax-embedded tissue sections with proven good reactivity. Negative controls were performed by a) omission of the primary antibody; b) substituting the respective primary antibody by non-immune normal mouse serum at different concentrations. Sections obtained from locations adjacent to the tumours, were used as normal control tissues. All incubations were carried out in humidified chambers to prevent evaporation.

Proliferative indices and quantitative analysis. Immunohistochemical analysis of proliferating cell nuclear antigen (PCNA) was used to demonstrate levels of cellular proliferation of thyroid tumours by the peroxidase, anti-peroxidase (PAP) method. Tumour sections were scanned in the entire area with a counting grid and for each field the number of points in coincidence with the area to be measured was recorded (point count method). The quantitative analysis of TRX, TRX-R and PCNA was carried out by calculating the percentage of stained cells in 10 low-power magnification fields or 16 high-power fields using a Zeiss microscope connected to a computer using image analysis software. To ensure reproducible and objective assessment of staining, 10 representative areas, each containing 1000 tumour cells, were observed under high-power field (objective lens, ×40) in a vertical section taken from the centre of the lesion. The proportion of positive cells was expressed as percentage of total cells counted.

Results

Clinico- and histopathological diagnostic evaluation of thyroid tumours. Histological examination and diagnosis of PTC is simple but has a limited value in tumours with follicular pattern, thus resulting in a preoperative diagnostic challenge. In such situations, immunohistochemistry may be of great value. The established prognostic factors of age, tumour size, prevalence of lymph node metastasis and histological classification of the carcinoma are of prognostic significance for survival in long term follow-up. The main criteria for the diagnosis of PTC is the orcurrence of ground-glass nuclei. These nuclei are enlarged, round to oval structures with pale karyoplasm condensing continously to the nuclear membrane due to cytoplasmic pseudo-inclusions. The nuclei are densely arranged and often overlap each other. Papillary carcinoma usually has papillae in its structure which are delicate stalks of epithelial cells situated on basal membranes covering stromal fibers and thin capillaries. Often the tumours contain round laminated calcifications (17). The diagnosis of follicular carcinoma is based on the histopathological demonstration of infiltrative growth. There are two criteria: i) true infiltration of the venous vessels outside the tumour capsule, and ii) fungus-like infiltration through the tumour capsule into the surrounding parenchyma by capsular infiltration, or better extra-capsular extension. Medullary thyroid carcinoma (MTC) is a calcitonin-producing tumour of the parafollicular C-cells, accounting for 5-10% of all thyroid tumours. The tumour is characteristically composed of solid nests and infiltrating formations of polygonal or spindle-shaped cells. Amyloid deposits within the stroma are found in about half of the tumours. The only effective treatment is the early and total surgical removal of all neoplastic tissue. The prognosis of patients with advanced MTC, with unresectable or distant metastases is poor, and chemotherapy or irradiation is of no significant value. Undifferentiated (anaplastic) carcinoma displays cytological polymorphism and histological dedifferentiation. Histopathologically, this tumour reveals solid sheets of highly anaplastic cells or spindle cells with morphologically sarcoma-like areas and frequent appearance of giant cells. Most tumours show large areas of necrosis and haemorrhage.

Association with reproductive factors. There was an association between age at first and last pregnancy and risk of thyroid cancer. Young age at first pregnancy seemed to have a protective effect, as the odds ratios were consistently below unity for ages 16-24 years. Women who had their last pregnancy at ages ≥30 years were at a significantly increased risk compared to those who had their last pregnancy at earlier ages. There was also a significant trend in risk with increasing age at last pregnancy. With regard to parity, the odds ratios were consistently above unity for women who have more than two live births. Women with ≥five births were at a modestly increased risk compared to those who had fewer children.

Expression of TRX. Compared to normal thyroid tissue cells, intensity of TRX immunoreactivity in the neoplasms of the thyroid was increased in both the nuclei and cytoplasm in the majority of cases investigated. Table I shows the clinicopathological characteristics and thioredoxin expression, obtained from counting a total of 1000 cells in each of 10 representative fields from each case of thyroid neoplasm investigated. Intensity of staining as a measure of TRX expression was present to varying degree in all tumour specimens and consistently showed prominent immuno-reactivity in malignant follicular-, papillary- and invasive follicular carcinoma tumour cells and to a lesser extend in the cells of the benign neoplasms. Expression of TRX was predominantly cytoplasmic, but strong nuclear immunoreactivity

was also apparent in many instances (Figures 1-3). Increased expression of TRX immunoreactivity was found in 15.1% of the 15 benign colloid nodule cases, in 38.0% of colloid goitres, in 43.4% of the 21 cases multinodular goitres, in 41.7% of the papillary oncocytic neoplasms, in 36.0% of the follicular adenomas cases, in 65.2% of the 24 follicular carcinomas cases, in 72.8% of the 48 cases of papillary carcinomas, in 69.5% of all 19 cases of invasive follicular carcinomas and in 79.6% of all medullary carcinomas investigated. The strong immunoreactivity exhibited with the anti-human thioredoxin antibody 2B1 is concordant with the specificity of this antibody. Tissue sections incubated with 20% normal serum, without the primary monoclonal antibody or with unrelated primary antibodies of identical isotype at the same concentration as the TRX antibody, did not display detectable immunoreactivity.

Expression of TRX-R. Increased levels of TRX immunoreactivity positively correlated with TRX-R expression and localization. This enzyme, involved in the reduction of TRX, was also highly expressed in cells of thyroid neoplasms, reflecting that a large amount of its TRX substrate is also present. Of the 166 cases of thyroid neoplasms investigated, overexpression of TRX-R was found in 12.4% of benign colloid, in 40.9% of colloid goitres, in 45.1% of multinodular goitres, in 36.6% of the papillary oncocytic neoplasms, in 32.8% of follicular adenomas tumour cells, in 58.0% of the follicular carcinoma cells, in 65.7% of the papillary carcinomas (Figure 4), in 71.6% of invasive follicular thyroid carcinomas and in 74.3% of medullary carcinoma tumour cells investigated.

PCNA. Table I shows the positonity for percentage of PCNA obtained from counting a total of 1000 cells in each of ten representative fields from each tumour specimen. Overnight application of primary anti-PCNA at a 1:50 dilution at 4˚C overnight in humidified chambers revealed distinct nuclear labelling and low background with diaminobenzidine (DAB) as the visualising agent. Somewhat superior staining results were achieved with 3-amino-9-ethyl carbazole (AEC) as the visualising chromogen. PCNA immunoreactivity was present in all cases investigated and varied from being more than 90% negative to a positive labelling of the great majority, with a variable proportion of stained cells in different thyroid tumours. A distinct to strongly positive staining intensity was present in the nuclei of these cells. Increased expression of PCNA immunoreactivity was found in 5.9% of the 15 benign colloid nodule cases, in 24.7% of all colloid goitres, in 44.3% of the 21 cases multinodular goitres investigated, in 15.9% of papillary oncocytic neoplasms investigated, in 39.0% of the follicular adenomas cases, in 47.8% of all 24 cases of follicular carcinomas cases investigated, in 51.2% of all 48 cases of papillary carcinomas, in 58.5% of the 19 cases of invasive follicular thyroid carcinomas, in 61.1% of all medullary carcinomas and in 65.4% of the anaplastic carcinomas investigated. The nuclei of normal tissue cells, including fibroblasts, vascular endothelial cells, adipocytes, smooth muscle cells, lymphocytes, neutrophills, mast cells and plasma cells were mostly negative.

Discussion

Thyroid carcinoma is a malignant representative of those originating from endocrine organs, arising from normal thyroid follicular cells. Follicular carcinoma is believed to originate from pre-existing follicular adenoma. On the other hand, no precursor lesions of papillary carcinoma have been identified. These carcinomas are generally slow growing with an excellent prognosis. However, once these carcinomas show anaplastic transformation (dedifferentiation), they display the most rapidly progressive character of all human malignancies. Cellular proliferation is a prominent parameter for evaluating the biological characteristics of carcinomas, and studies of positive and negative regulators of the cell cycle have been performed in various human tumours (20-22). The morphological characteristics betweem follicular adenoma and follicular carcinoma are similar and hence follicular carcinoma is believed to occur from benign adenoma. Problems with observer variations can lead to low diagnostic reproduceability, especially in the diagnosis of FTC. The oxyphilic-cell type of papillary carcinoma causes diagnostic problems because it obscures the pattern of ground-glass nuclei. The follicular variant of papillary thyroid carcinoma still behaves biologically as papillary carcinoma, provided it meets the nuclear criteria for diagnosis of papillary cancers. The true follicular carcinoma, in contrast, lacks these nuclear features, frequently demonstrates capsular and vascular invasion, and has a less favourable prognosis. Thus, the two need to be properly differentiated with regard to their prognosis and management.

Two established discriminatory immunohistochemical markers for thyroid tumours with follicular and parafollicular origin are thyroglobulin and calcitonin, respectively. Thyroglobulin is present in more than 95% of papillary and follicular carcinomas. Poorly differentiated carcinomas contain less thyroglobulin than do more differentiated tumours. Anaplastic carcinomas are most immunogenic for thyroglobulin. The great majority of medullary carcinomas are positive for calcitonin. The vast majority of papillary carcinomas are cytokeratin type 19 immunopositive. However, cytokeratin 19 is also present in other thyroid tumour types (19). Thyroid transcription factor-1 (TTF-1) and gal-3 (23) are also some of the potential candidates as tumour markers in differentiating thyroid malignancies that are difficult to confirm in routine histopathology.There is direct clinical evidence for the role of hormonal factors in thyroid cancer to suggest that hormonal and biochemical

changes related to reproductive events and patterns could be relevant to the etiology of benign thyroid dysfuntiion or disease and neoplasia. Elevated levels of thyroid stimulating hormone (TSH) are associated with thyroid hyperplasia and elevate the risk of neoplastic transformation. Increased secretion of TSH occurs during puberty, pregnancy, delivery and puerperium, as well as while using oral contraceptives (24). In healthy pregnant women, there is a progressive increase in TSH levels, basal metabolic rate and thyroid volume (pseudo-goitre) throughout pregnancy. Serum human chorionic gonadotrophin, with its marked elevation in early pregnancy, may directly stimulate the thyroid gland through its TSH-like activity (25).

Thioredoxin and thioredoxin reductase are widely distributed, but vary considerably between different cell types. For instance, hepatocytes, epithelial cells, secretory cells, plasma cells and neurons display high contents of immunoreactive TRX and TRX-R, while mesenchymal cells of connective tissues, bone, muscle and fibrous tissue, in contrast, show low or no immunoreactivity. TRX and TRX-R have been identified in the cytoplasm, nuclei, cell membrane and extra-cellularly. In the cytoplasm they exhibit antioxidant and cofactor activity; in the nucleus they are involved in the regulation of transcription factor activity; in the mitochondria they associate with isoenzymes and extracelluarly they stimulate cell growth and chemotactic activity.

A number of cancer cells are known to secrete TRX in varied amounts (8, 11, 12, 14). The results from this immunohistochemical analysis show that both TRX and TRX-R, compared to their normal tissue counterparts, are both overexpressed in the nuclei and cytoplasm of the majority of thyroid neoplasms (Table I, Figures 1-4). Overexpression of TRX indicates the possible involvement of TRX in the process of oncogenesis. In cancer cells, the secreted TRX tends to sensitize the cells to growth factors, produced by the cancer cells themselves (26), by increasing the potentiating effect of growth factors, making the cells more susceptible to them and therefore leading to increased cell growth. TRX therefore enhances growth factors and stimulates the growth of neoplastic cells. In MCF-7 breast cancer cells, the effects of interleukin-2 (IL-2) were increased up to 1000 fold and that of basic fibroblast growth factor up to 100 fold, providing evidence that TRX potentiates the growth stimulation of growth factors (27). Plasma and serum levels of TRX in normal healthy individuals are between 0.8 to 6.6 nM, but this level is greatly increased in tumours and can be used as a marker (28). TRX has been shown to act as a chemotactic protein causing the migration of neutrophils, monocytes and T-cells with a potency similar to known chemokines, including interleukin-8 (IL-8). Moreover, it has some cytokine like activities such as regulating eosinophil migration and promoting lymphoid cell growth. It differs only from other chemokines in one respect, being G-protein independent (29). Extracellular TRX participates in the regulation of cell to cell contact and contributes to redox regulation in the cell extracellular space.

Table I. *Expression of thioredoxin (TRX), thioredoxin reductase (TRX-R) and proliferating cell nuclear antigen (PCNA) in neoplasms of the human thyroid gland.*

Tumour type	Number of patients	PCNA* % +ve	TRX* % +ve	TRX-R* % +ve
Benign colloid nodule	15	5.9	15.1	12.4
Colloid goitre	14	24.7	38.0	40.9
Multinodular goitre	21	44.3	43.4	45.1
Papillary oncocytic neoplasm	10	15.9	41.7	36.6
Follicular adenoma	16	39.0	36.0	32.8
Follicular carcinoma	24	47.8	65.2	58.0
Invasive follicular carcinoma	19	58.5	69.5	71.6
Papillary carcinoma	48	51.2	72.8	65.7
Medullary carcinoma	9	61.1	79.6	74.3
Anaplastic carcinoma	7	65.4	78.9	81.5

*Compared to corresponding normal tissue.

TRX-R, the enzyme involved in the reduction of TRX, was also present in large amount in the thyroid cancer tissues, reflecting that a large amount of its substrate (TRX) is also present. Reduced TRX acts as a cofactor for ribonucleotide reductase which catalyzes the transfer of ribonucleotides to deoxribonucleotides for DNA synthesis. It is involved in gene transcription and modulates gene regulation by acting directly on several transcription factors mediating redox control of critical thiol groups, including regulation of NF-κB transcription complex important for tumourigenesis and several other transcription factors, such as TFIIIC, glucocorticoid and estrogen receptors (30).

Reduced TRX also acts as a structural component by binding with high affinity to DNA polymerase to form a complex, which in turn gives the enzyme its high processing capacity and makes an inhibitory complex with apoptosis signaling kinase 1 (ASK1), providing the means of redox regulation of apoptosis (31). When the oxidative stress rises in the cell, the antioxidant effect of TRX comes into action, reducing the stress (32). A rise in cellular oxidative stress causes induction of apoptosis, whereas reduced oxidative stress inhibits apoptosis. The rise in oxidative stress, inducing apoptosis, is lowered by TRX, triggering a fall in oxidative stress (inhibition of apoptosis). Moreover, overexpression of TRX peroxidase, acting as antioxidant, is also related to apoptosis, the overexpression tending to reduce H_2O_2 levels, causing inhibition of the induction of apoptosis (33). Selenium is an essential factor of TRX-R and increased selenium incorporation translates to increased enzyme activity. Increase in the TRX-R activity shows no effect on cell growth, while a reduction which results in the reduction

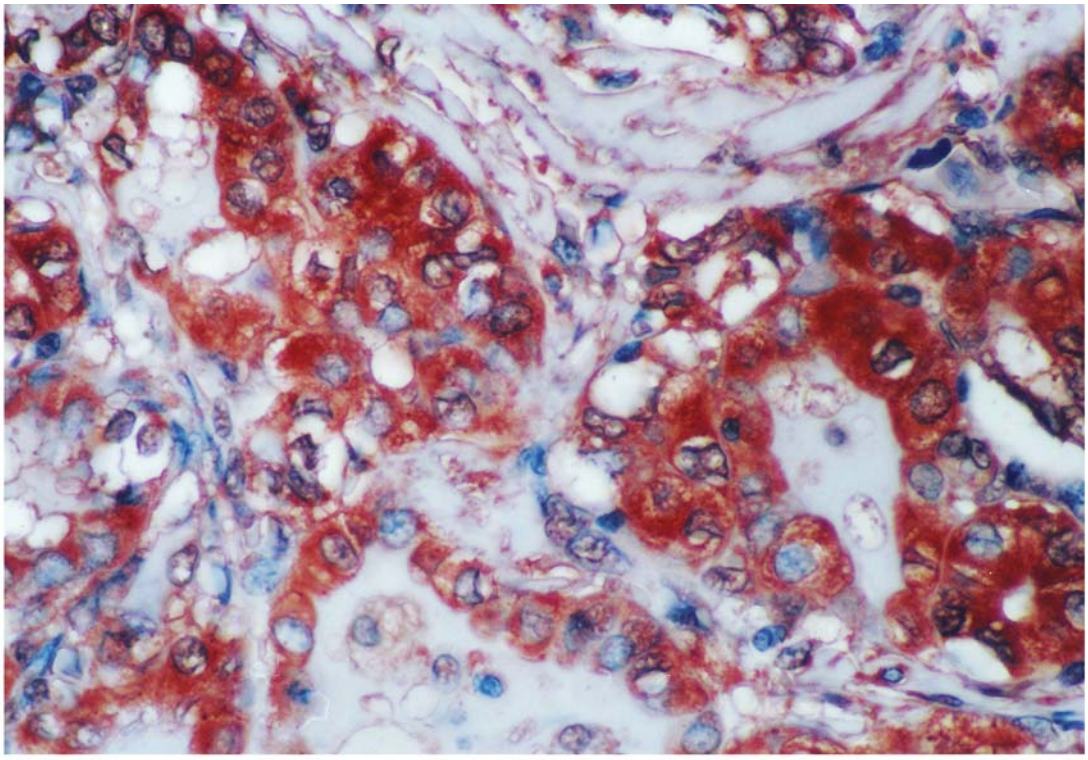

Figure 1. *Thioredoxin expression in a papillary carcinoma of the thyroid from a 56-year-old female patient (2647/98), using MAb 6F3 and visualisation with 3-amino-9-ethylcarbazole.*

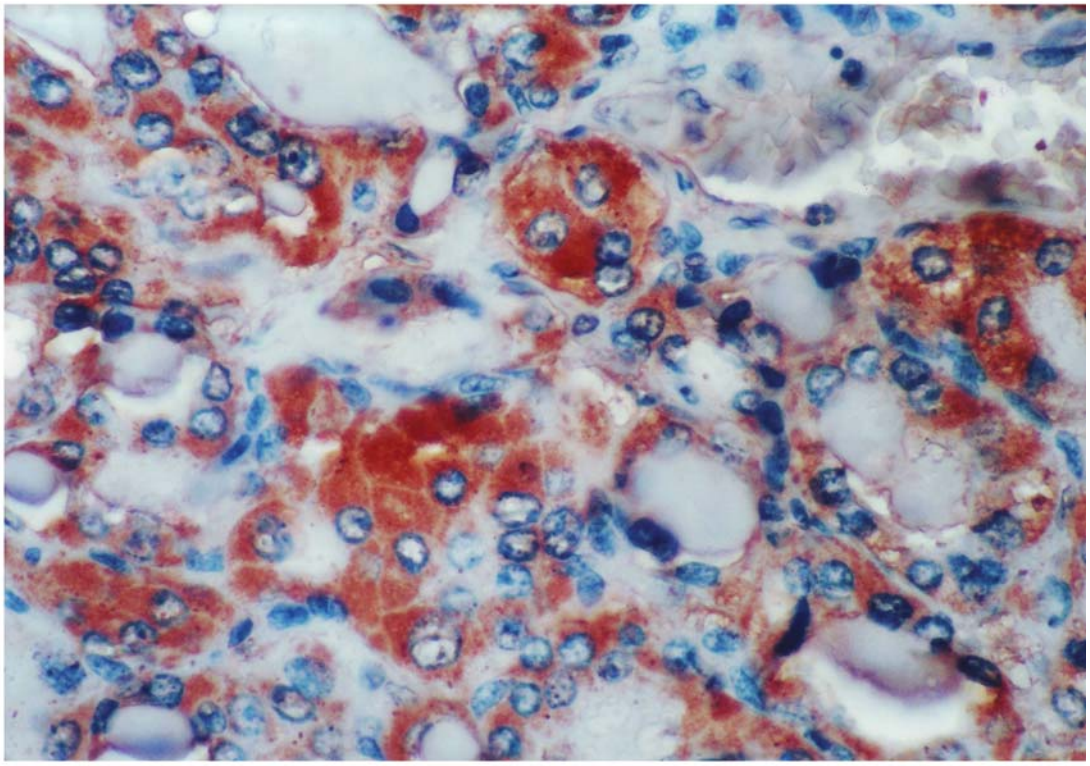

Figure 2. *Thioredoxin expression in a follicular carcinoma of the thyroid from a 60-year-old male patient (2395/95), using MAb 6F3 and visualisation with 3-amino-9-ethylcarbazole.*

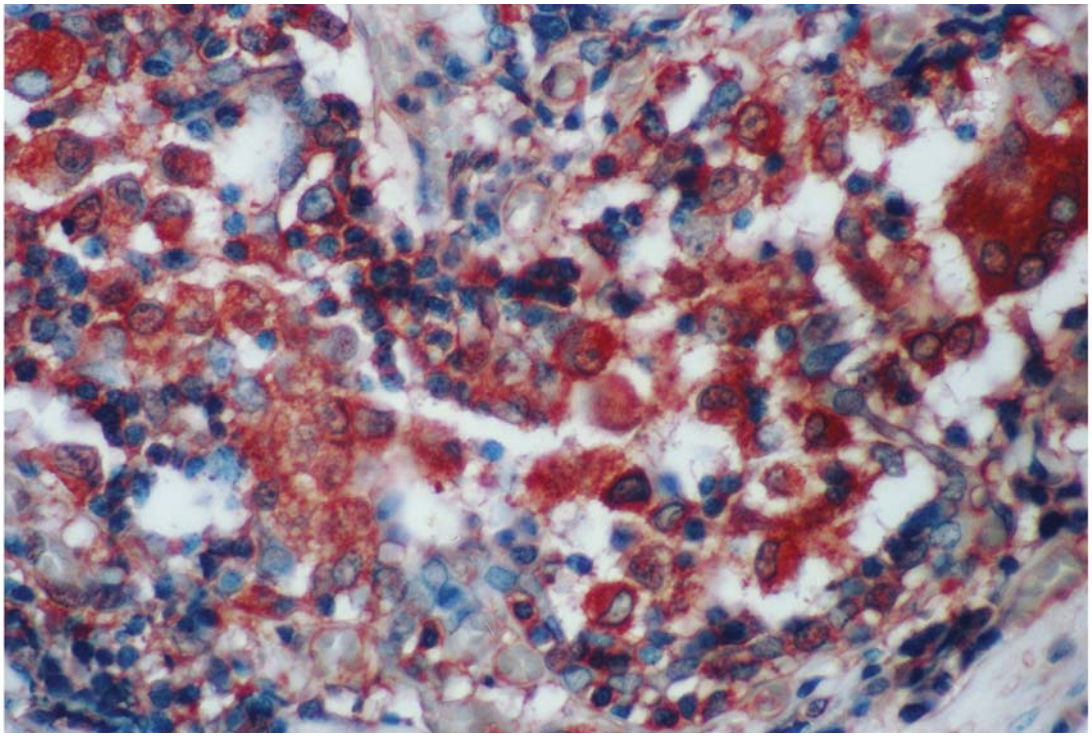

Figure 3. *Thioredoxin expression in a medullary carcinoma of the thyroid from a 39-year-old female patient (1925/98 B1), using MAb 6F3 and visualisation with 3-amino-9ethyl-carbazole.*

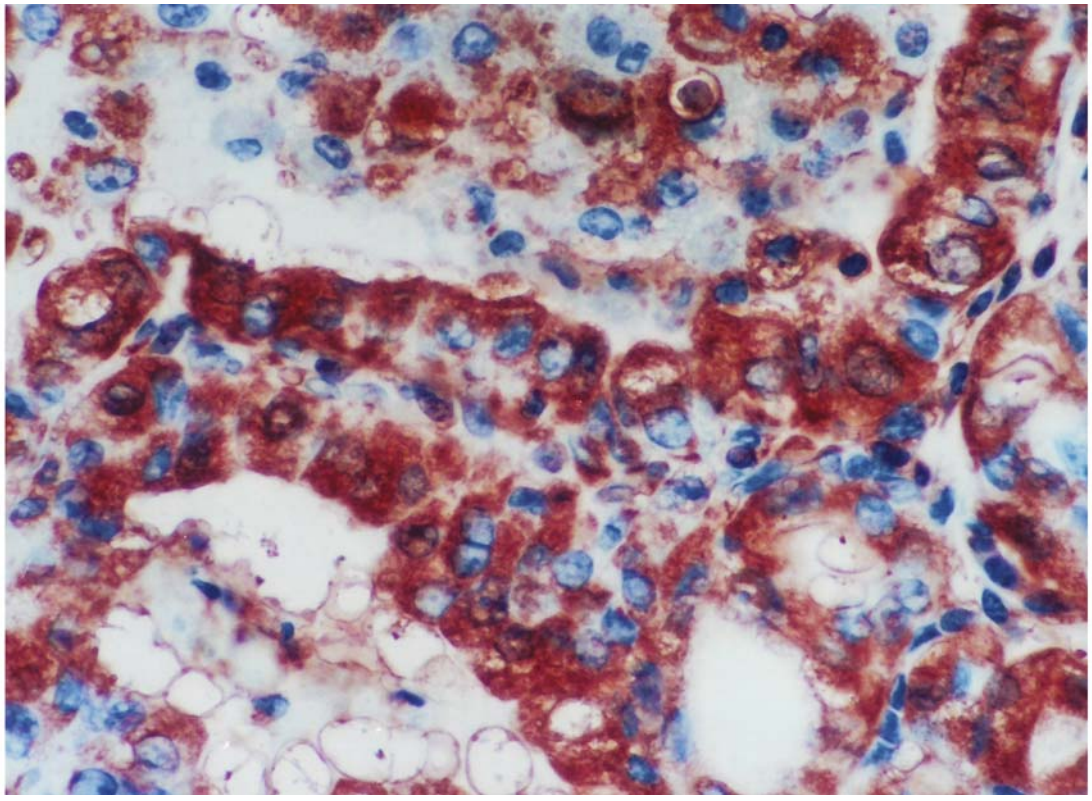

Figure 4. *Thioredoxin reductase expression in papillary carcinoma of the thyroid from a 56-year-old female patient (166/02 A2-3). Rabbit anti-human thioredoxin reductase gamma G fraction. Visualisation with 3-amino-9-ethylcarbazole.*

of the reduced form of TRX and therefore a reduction in reducing equivalents due to inhibition, causes decreased cell growth. Reduced TRX increases cell growth by increasing the supply of reducing equivalents for DNA synthesis, resulting in cell proliferation and activation of transcription factors that regulate cell growth, thereby increasing the sensitivity of cells to growth factors and cytokines (18).

This immunohistochemical investigation of a variety of thyroid neoplasms shows that TRX is increased in more than half of the tumours, compared to levels in the corresponding normal tissue. The results indicate that tumour cells use TRX as an autocrine growth stimulate. Overexpression of TRX indicates its possible involvement in the process of oncogenesis. Occurrence of oxidative stress within the cell induces TRX-R release, which, due to its anti-apoptotic activity, causes inhibition of apoptosis, resulting in abnormal cell proliferation which can intiate cancer development. The TRX levels have been reported to be positively correlated ($p<0.001$) with cell proliferation measured by nuclear proliferation antigen and a highly significant negative correlation with apoptosis measured by the terminal deoxynucleotidyl transferase assay (34). In a immunohistochemical study of TRX-1 levels in human colon cancer, TRX-1 protein was not increased compared to normal colonic mucosa in precancerous colon polyps but was increased six-fold in primary colon tumours and almost nine-fold in metastatic colon tumours in adjacent lymph glands (35). Furthermore, high levels of TRX-1 in the tumour appear to be associated with decreased patient survival.

Conclusion

We propose that one function of extracellular TRX secreted by cancer cells is to facilitate the mechanisms which give expression to the invasive phentoype. While a normal physiological example of this function is the implantation of embryos in the uterus, it is likely that cancer cells exploit this function of TRX to enhance their invasive capacity. This argument is supported by the following facts: i) TRX and TRX-R, compared to normal tissues, are both overexpressed in the nuclei and cytoplasm of the majority of thyroid neoplasms; ii) TXR and TXR-R are localised in the cytoplasm, nuclei, cell membrane and extracellularly. In the cytoplasm, they exhibit antioxidant and cofactor activity; in the nucleus they are involved in the regulation of transcription factor activity; in the mitochondria they associate with isoenzymes and extracelluarly they stimulate cell growth and chemotactic activity; iii) Compared to levels in the corresponding normal tissue, TRX is increased in more than half of all thyroid neoplams investigated. The results indicate that thyroid tumour cells use TRX as an autocrine growth stimulate. Overexpression of TRX indicates the possible involvement in the process of oncogenesis; iv) In tumour cells, the secreted TRX tends to sensitize the cells to growth factors, produced by tumour cells themselves, by increasing the potentiating of the growth factors, making the cells more susceptible to them and therefore leading to increased cell growth. TRX therefore enhances growth factors and stimulates the growth of neoplastic cells; v) TRX-R, the enzyme involved in the reduction of thioredoxin, was also present in large amount in thyroid neoplasms, reflecting that a large amount of its substrate (TRX) is also present; and vi) Occurrence of oxidative stress within the cell induces TRX-R release, which, due to its anti-apoptotic activity, causes inhibition of apoptosis, resulting in abnormal cell proliferation which can intiate cancer development.

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