Mammalian stanniocalcins and cancer

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Abstract
Stanniocalcin (STC) is a glycoprotein hormone that is secreted by the corpuscle of Stannius, an endocrine gland of bony fish, and is involved in calcium and phosphate homeostasis. The related mammalian proteins, STC1 and STC2, are expressed in a wide variety of tissues. The ovaries have the highest level of STC1, and this increases during pregnancy and lactation. STC1 is present in breast ductal epithelium, and its expression is induced by BRCA1, a tumor suppressor gene that has an important role in breast and ovarian cancer. The expression of STC2 is induced by estrogen, and there is a positive correlation between the level of expression of estrogen receptor and expression of both STC1 and STC2 in breast cancer. This article reviews the data currently available regarding the mammalian STCs, and discusses the roles they may play in normal physiology and in breast and other cancers.

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Introduction
Stanniocalcin (STC) derives its name from the corpuscle of Stannius (CS), an endocrine gland associated with the kidneys of fish (Stannius 1839). CS extract (Fontaine 1964) and purified STC (Lafeber et al. 1988a) were found to have an anti-hypercalcemic effect due to inhibition of whole-body Ca$^{2+}$ influx. The principal target organs for this effect are the gill (Wagner et al. 1988) and gut (Takagi et al. 1985, Sundell et al. 1992). STC was also found to stimulate resorption of inorganic phosphorus (P) by proximal tubule epithelium cells from fish kidney (Lu et al. 1994).

A human ortholog of fish STC, STC1, was found by mRNA differential display of genes related to cellular immortalization, a key aspect of the cancer cell phenotype (Chang et al. 1995), and independently by random sequencing of a fetal lung cDNA library (Olsen et al. 1996). An STC1 paralog, STC2, was identified by searching for related sequences in expressed sequence tag (EST) databases (Chang & Reddel 1998, DiMattia et al. 1998, Ishibashi et al. 1998). Although mammalian STC1 and STC2 are not expressed ubiquitously, they are expressed in a wide variety of tissues, including endocrine glands and hormone-responsive organs. The ovaries appear to have the highest STC1 levels out of all the tissues examined to date with increased expression observed during pregnancy and lactation (Deol et al. 2000). STC1 is also found in breast ductal epithelium (Welch et al. 2002).

There is now mounting evidence that altered expression of the STCs may have a role in human cancer. cDNA microarray analyses showed that STC1 is upregulated in most primary hepatocellular carcinomas (Okabe et al. 2001). STC1 is downregulated 7-fold in ovarian cancer compared with normal ovarian epithelial cells (Ismail et al. 2000). An anonymous cDNA fragment shown to be downregulated in a breast cancer cell line (Liang et al. 1992) was subsequently identified as a portion of the 3′ untranslated region (UTR) of STC1 (Chang et al. 1995). However, STC1 and STC2 are both expressed in estrogen receptor (ER)-positive breast cancer (Bouras et al. 2002). Expression of STC2 is inducible by estrogen (Charpentier et al. 2000, Bouras et al. 2002) and repressed by anti-estrogen (Bouras et al. 2002). Expression of STC1 is upregulated by the tumor suppressor gene BRCA1, mutations of which predispose to breast and ovarian cancer, and loss of BRCA1 and STC1 expression are correlated in breast cancer (Welch et al. 2002). There is interest in the possible use of STC1 and/or STC2 expression for diagnosis and/or classification of breast cancer.

This article reviews our current understanding of the mammalian STCs and their possible roles in breast and other cancers.

Identification of mammalian STC genes
As the CS gland has only been found in holostean and telostean fish, it had long been supposed that STC did not exist in other vertebrates (Wendelaar Bonga & Pang 1991). The first suggestion that there might be a mammalian STC was based on demonstrating STC immunoreactivity in the sera of...
several species including human and in human kidney (Wagner et al. 1995), although a later study using more specific antibodies failed to detect STC in human sera (De Niu et al. 2000). Definitive evidence for the existence of human STC was provided by Chang et al. (1995), who identified a cDNA that was downregulated following immortalization of SV40-transformed human fibroblasts. When the cDNA was sequenced, the predicted amino acid sequence was found to share ~60% identity and ~73% similarity with various fish STCs. Due to its high degree of homology to fish STC, this novel mammalian protein was also named STC (Chang et al. 1995). When a second member of this gene family was identified, mammalian STC was renamed STC1. The same gene was independently isolated by Olsen et al. (1996) during random sequencing of an early-stage human fetal lung cDNA library.

A second human and mouse STC gene, STC2, was identified by searching EST databases for sequences related to STC1 (Chang & Reddel 1998). Subsequently, the STC2 gene was independently identified using the same methodology by Ishiashi et al. (1998) as well as by DiMattia et al. (1998) who referred to the protein as ‘STC-related protein’.

**STC1 and STC2 proteins**

The human STC1 cDNA encodes a protein of 247 amino acids (Fig. 1). The level of sequence similarity to salmon STC is 92% over the first 204 amino acids, 118 residues of which are identical. However, the last 43 residues at the C-terminus are completely divergent. The human STC2 cDNA encodes a protein of 302 amino acids that has 34% identity to both STC1 and eel STC (Fig. 1). The relatedness of STC2 to STC1 and eel STC is greatest at the N-terminus, residues 41–160 being 40% identical. There is a greater similarity level, 53%, between STC1 and eel STC. Clearly, STC1 is more closely related to fish STC than to STC2.

As with fish STC, mammalian STC1 and STC2 are predicted to be secreted glycosylated proteins with a signal peptide sequence of about 24 amino acids and a pro-sequence of about 15 amino acids that are subsequently processed to yield the mature proteins (Moore et al. 1999). A strikingly conserved feature of STCs is the presence and location of cysteine residues. There are 11 cysteine residues in STC1 with the same spacing as those in eel and coho salmon STC (Butkus et al. 1987, Wagner et al. 1992). This odd number of cysteine residues is consistent with STC1 being a homodimer in the native state, with the cysteines participating in interchain and intrachain disulfide bonding as in STC (Laferber et al. 1988b). Disulfide linkages in chum salmon STC have been defined (Hulova & Kawauchi 1999). Chum salmon STC is a homodimer connected by a single intermonomeric disulfide bond at Cys169. The monomer contains 179 amino acids, with five intramonomeric disulfide bonds formed between Cys12-Cys26, Cys21-Cys41, Cys32-Cys81, Cys65-Cys95 and Cys102-Cys137. STC2 has 15 cysteines, of which ten have the same spacing as STC and STC1 (Fig. 1), and also exists as a disulfide-linked homodimer (Moore et al. 1999).

The N-linked glycosylation site is also conserved (Fig. 1). The consensus sequence, Asn-X-Thr/Ser (around residues 62–72), is believed to be glycosylated in eel and coho salmon (Butkus et al. 1987, Wagner et al. 1992); however, direct sequence data are lacking. STC1, on the other hand, was analyzed to determine the saccharide content in both baculovirus and Chinese hamster ovary (CHO) cell expression systems (Zhang et al. 1998). At least one N-linked site was found to be glycosylated and no O-linked oligosaccharides were found.

An unusual feature of STC2 is the presence of 15 histidine residues. This is five times the number seen in eel STC and more than twice the number in STC1. Four such residues are clustered towards the C-terminus of STC2 and possibly interact with transition metals. Evidence for this interaction has been obtained by Moore et al. (1999) who utilized a nickel chelating column to purify STC2.

**Chromosomal localization and genomic structure**

The human STC1 gene is on the short arm of chromosome 8 (8p11.2–p21). The STC1 gene contains four exons spanning 13 kb, and transcriptional start sites have been localized 284, 271 and 153 nucleotides 5’ of the initiator methionine codon (Chang et al. 1998, Varghese et al. 1998).

The 5’ UTR is rich in the trinucleotide repeat, CAG. This area usually has 19 such repeats clustered within 102 nucleotides of the transcription start site, but is polymorphic (Chang et al. 1998). Trinucleotide repeats are associated with a number of genetic conditions including neurodegenerative diseases (Reddy & Housman 1997). In the case of STC1, there are four relatively small blocks, each consisting of three to six CAG repeats separated by 6–15 nucleotides. The intervening sequences, however, are 72% GC-rich and contain six CGG triplets. This region may represent a transcriptional control domain.

The STC2 gene has been localized to chromosome 5q33 or 5q35 (White et al. 1998, Moore et al. 1999), and also contains four exons (Ishibashi et al. 1998). The exon–intron boundaries are fully conserved between STC2 and STC1, indicative of a common ancestral gene. In contrast to STC1, no CAG repeats are found in the 5’ or 3’ UTR of STC2.

The fish STC gene, about 4 kb long, has been isolated from sockeye salmon (McCudden et al. 2001a), and contains five exons. From comparative alignment of the gene structures (Fig. 2), it can be seen that the size of exon 2 is highly conserved. Mammalian exon 3 corresponds to exons 3 and 4 in fish, so the mammalian exon 3 most likely represents a fusion of the fish exons 3 and 4. The existence of a fish STC2 variation system (Zhang et al. 1998).
**Figure 1** Comparison of fish STCs with human STC1 and STC2. The alignment of amino acid sequences of STCs from coho salmon (*Oncorhynchus kisutch*; GenBank accession #I51197), and Australian eel (*Anguilla australis*; accession #P18301) with human STC1 (accession #P52823) and STC2 (accession #O76061) was performed by the clustal program (http://www.align.org.au). Shading intensity correlates with the degree of homology, with the darkest shading indicating 100% identity. The putative N-linked glycosylation site is boxed. The ten cysteine residues are asterisked to highlight their spatial conservation and the cluster of four histidines in STC2 is double underlined.

gene has not been reported, but preliminary analysis of the pufferfish genome sequence (www.fugubase.org) indicates that it contains at least one STC2 gene. This suggests that mammalian STC1 and STC2 most likely arose from corresponding fish homologs.

### STC1 mRNA and protein expression

STC1 mRNA is expressed in a variety of human and mouse tissues (Chang et al. 1995, Olsen et al. 1996, Varghese et al. 1998). In the human, STC1 is expressed predominantly as a 4 kb transcript and the tissues with highest expression include the kidney, ovary, prostate and thyroid. In the mouse, a transcript of 4 kb is also detected and the kidney, ovary and pituitary are among those with highest expression. The existence of mammalian STC1 mRNA in a wide variety of tissues was regarded as strikingly different to fish STC, as the latter was thought to be expressed exclusively in the unique endocrine gland, the CS.

These observations led to a re-examination of fish STC gene expression (McCudden et al. 2001a). Northern blotting of poly(A+) RNA detected STC mRNA in testis, ovary and anterior kidney at levels about 100-fold lower than in the CS, which may explain why they had been overlooked previously. The presence of STC mRNA in gonadal tissues suggests that fish STC may have a role in reproduction. This is supported by evidence that STC is preferentially expressed in early-stage oocytes, with levels diminishing as oocytes develop (McCudden et al. 2001b). Another group has also reported STC localization in renal tubules (Amemiya et al. 2002). It is unclear at present whether STC produced in sites apart from the CS has a local action or is released systemically as from the CS.

The wide tissue distribution of mammalian STC1 mRNA is strongly suggestive of an autocrine or paracrine role.
Circulating STC1 is usually not detected, except in pregnancy (Deol et al. 2000). An RIA for STC1 did not detect STC1 in the blood of rats, sheep, goats, horses or pigs (De Niu et al. 2000). Potential target tissues of STC1 were determined following i.v. injection of $^{125}$I-STC1 into the rat, with the highest accumulation seen in kidneys, spleen and small intestine (De Niu et al. 2000). It has been subsequently concluded, however, that iodination of STC1 damages its binding activity, and studies with an STC1-alkaline phosphatase fusion protein detected the highest number of STC1 binding sites in the liver and kidneys (McCudden et al. 2002).

Detailed analyses of STC1 mRNA expression and protein localization have been reported for tissues including kidneys, ovaries, neurons, bone and muscle.

Kidneys

STC1 protein was detected in all proximal straight tubule cells, cortical thick ascending limb cells, distal convoluted tubule cells, and in both principal and $\alpha$-intercalated cells of rat kidney (Wong et al. 1998). The distribution of STC1 mRNA, however, did not parallel STC1 immunoreactivity. In situ hybridization revealed STC1 mRNA expression only in cortical and medullary collecting duct cells. These findings suggest that STC1 is synthesized and secreted by the collecting ducts and accumulates in other areas of the kidney, consistent with evidence that proximal tubules are an STC1 target (Haddad et al. 1996, Olsen et al. 1996, Worthington et al. 1999). STC1 localization in the kidney has been reviewed recently in more detail (Ishibashi & Imai 2002). The expression of STC1 in the distal half of the nephron is consistent with a regulatory role in renal Ca$^{2+}$ handling. As discussed below, STC1 may also play a role in the adaptation of kidney cells to osmotic stress (Sheikh-Hamad et al. 2000).

The kidneys of STC1-transgenic mice weighed more than those of age-matched controls, suggesting that STC1 may have a trophic effect on the kidney (Filvaroff et al. 2002). In a study of STC1 expression during mouse kidney development, STC1 was found to be produced by undifferentiated mesenchymal cells between embryonic days 10.5 and 14.5 and sequestered by non-expressing ureteric bud epithelial cells (Stasko & Wagner 2001b). This suggests that STC1 might have a role in mesenchymal–epithelial signaling.

Ovaries

Of all tissues studied to date the ovaries have the highest STC1 mRNA levels, 5- to 10-fold higher than in kidneys (Varghese et al. 1998). Mouse ovarian STC1 levels increased during gestation and lactation (Deol et al. 2000). STC1 secretion by rat and bovine thecal-interstitial cells was increased by human chorionic gonadotropin (hCG), most likely via cAMP-mediated activation of protein kinase A (Paciga et al. 2002). The secreted STC1 protein is substantially larger than the $\sim$50 kDa homodimer produced by other tissues. The size of these higher molecular mass variants (dubbed ‘big STC’) has not been explained, but does not appear to be due to differential glycosylation (Paciga et al. 2002). STC1 protein was found in oocytes and corpus luteal cells, but STC1...
mRNA expression was found in secondary interstitial and theca interna cells (Varghese et al. 1998). Thus, like the kidney, the ovary also has STC1-producing and STC1-sequestering cells, suggesting that STC1 has a cell–cell signaling role. The timing of STC production is consistent with STC1 exerting a prosurvival effect on the dominant ovarian follicle (Paciga et al. 2002).

Bone

CS extracts, like parathyroid hormone (PTH), can cause osteoclastic resorption of embryonic mouse bone in vitro (Lafeber et al. 1986). Evidence that STC1 may also be involved in bone physiology was obtained by detecting STC1 mRNA in neonatal mouse calvaria, primary cultures of mouse osteoblasts, and human and mouse osteoblastic cell lines (Yoshiko et al. 1999). STC1 mRNA expression was confined to osteoblasts and chondrocytes and was not present in osteoclasts and other bone marrow elements in both femur and calvaria. There is suggestive evidence from STC1-overexpressing transgenic mice, however, that STC1 might affect function of osteoclasts as well as osteoblasts and chondrocytes. STC1 transgenics had increased cartilage matrix and decreased bone length (Filvaroff et al. 2002). The rate of mineral deposition by osteoblasts but not the extent of bone mineralization was decreased, and it seemed that the rate of osteoclastic resorption was concomitantly decreased (Filvaroff et al. 2002).

In addition, studies of STC1 expression during early mouse development have shown that it is predominantly localized to cells in the developing muscle and bone (Jiang et al. 2000, Stasko & Wagner 2001a). In endochondral bone formation, it was found in prechondrocytes and prehypertrophic chondrocytes (Jiang et al. 2000). During intramembranous bone formation, it was localized to the mesenchyme that is about to engage in ossification. There were abnormalities in skulls and long bones of STC1-transgenic mice (Filvaroff et al. 2002). Therefore, on the basis of developmental and transgenic studies, STC1 has a role in both intramembranous and endochondral bone formation.

Neurons

Neuronal tissue is highly susceptible to Ca\(^{2+}\) fluctuations, as changes in extracellular Ca\(^{2+}\) alter membrane potential which affects both electrical activity and signal transduction. STC immunoreactivity has been detected in snail ganglia (Wendelaar Bonga et al. 1989), and platyfish brain (Fraser et al. 1991). Zhang et al. (1998) demonstrated the presence of STC1 in human and mouse neurons; STC1 immunoreactivity was present in fully differentiated neurons but not in immature brain neurons from fetal or newborn mice. A study of the differentiation of a mouse neuroblastoma cell line concluded that STC1 is involved in neuronal differentiation and, more specifically, has a possible role in axonogenesis (Wong et al. 2002).

STC1 was localized to pyramidal cells of the cerebral cortex and hippocampus as well as Purkinje cells of the cerebellum. These neurons are highly sensitive to ischemia, and a correlation between STC1 expression and hypoxic damage has recently been demonstrated (Zhang et al. 2000). Paju cells, derived from human neural crest, transfected with STC1 displayed increased resistance to both hypoxic stress and elevated intracellular Ca\(^{2+}\). In addition, an upregulation and redistribution of STC1 expression around brain infarcts was observed in human and rat parietal cortex. These results suggest that STC1 expression may protect the cell against hypoxic damage and Ca\(^{2+}\)-mediated cell death.

Muscle

High levels of STC1 protein were found in myocardiocytes of the developing mouse heart and at all stages of differentiation from myoblasts to myotube formation in developing skeletal muscle (Jiang et al. 2000). Transgenic mice overexpressing STC1 in muscle had smaller muscles, by actual weight and as a proportion of overall body mass, thanagematched control mice, and the muscle mitochondria were greatly enlarged (Filvaroff et al. 2002). The mice had a lower body mass, but had increased food and oxygen consumption and faster glucose clearance than control mice, suggesting that STC1 can regulate metabolic rate (Filvaroff et al. 2002), possibly through its effects on mitochondria, the location of high-affinity receptor-like STC1 binding activity (McCudden et al. 2002).

Other tissues

Other tissues of interest include the liver, which has an undetectable level of STC1 expression (Chang et al. 1995, Varghese et al. 1998) but has a high level of receptor-like activity in a subset of hepatocytes (McCudden et al. 2002). STC1 expression is generally low or absent in hematopoietic tissues (Chang et al. 1995), but mature megakaryocytes, which, like neurons, are terminally differentiated cells with extended survival, and platelets have a high STC1 content (Serlachius et al. 2002). STC1 appears to be involved in uterine remodeling following implantation. In the non-pregnant mouse uterus, STC1 mRNA and protein were found mainly in luminal epithelium (Stasko et al. 2001). On day 5 of pregnancy blastocyst implantation was accompanied by a shift in STC1 mRNA expression from the epithelium to adjacent stromal cells, but most of the STC1 protein was found in the epithelial cells (Stasko et al. 2001). This pattern of expression in one cell type and accumulation in another resembles the situation in ovary and kidney, and suggests that STC1 is involved in mesenchymal–epithelial signaling in the mouse uterus.
STC1 and mineral homeostasis

The high level of similarity of fish STCs (>70% of amino acid residues) to mammalian STC1 has focused interest on the possible role of STC1 in Ca\(^{2+}\) and Pi homeostasis. Recombinant STC1 inhibited uptake of Ca\(^{2+}\) through the gills when injected into goldfish, and stimulated renal Pi resorption and reduced renal Pi excretion when administered to rats (Olsen et al. 1996, Wagner et al. 1997). A similar effect of fish STC was demonstrated in monolayer cultures of winter flounder proximal tubule epithelium, where STC caused a dose-dependent stimulatory effect on net Pi resorption via a cAMP-dependent pathway (Lu et al. 1994). Isolated renal cortical brush-border membrane vesicles from STC1-treated rats had a 40% higher rate of Na\(^+\)/Pi cotransport when compared with vesicles from non-treated animals. In this study, STC1 had little effect on Ca\(^{2+}\), however, as there were no major changes in either plasma Ca\(^{2+}\) concentration or urinary Ca\(^{2+}\) excretion (Wagner et al. 1997). STC1 decreased absorption of Ca\(^{2+}\) and stimulated absorption of Pi, in both swine and rat intestine in Ussing chambers (Madsen et al. 1998). The expression of STC1 in choroid plexus epithelium is consistent with the notion that STC1 may have a role in regulation of Ca\(^{2+}\) and Pi levels in cerebrospinal fluid (Franzén et al. 2000).

Further evidence for a role of STC1 in mineral homeostasis has been provided by two transgenic mouse models in which STC1 overexpression was driven by either the mouse metallothionein 1 promoter (Varghese et al. 2002) or the muscle-specific rat myosin light chain 2 promoter (Filvaroff et al. 2002). The transgenic mice were small, and female transgenic mice gave birth to litters of reduced size, but male fertility was unaffected. The transgenic mice were found to have elevated serum Pi levels indicating that overexpression of STC1 perturbs mineral homeostasis. This effect on serum Pi, is consistent with the evidence that STC1 can stimulate Pi uptake by both kidney and gut. Circulating STC1 was detectable in the transgenic mice (Varghese et al. 2002), but is normally detectable in mice only during gestation (Deol et al. 2000). The role of STC1 in mineral homeostasis under normal conditions therefore awaits clarification.

Regulation of STC1 mRNA expression

Consistent with its role as an anti-hypercalcemic hormone, the main stimulus for STC secretion and degranalization of the CS cells in fish appears to be an increase in plasma Ca\(^{2+}\) levels (Aida et al. 1980, Lopez et al. 1984, Lafeber & Perry 1988, Hanssen et al. 1989, 1991). It has recently been shown that the Ca\(^{2+}\)-stimulated STC secretion is mediated by a Ca\(^{2+}\) ion-sensing receptor (Radman et al. 2002). In addition to triggering STC secretion, elevated extracellular Ca\(^{2+}\) increases STC mRNA in cultured CS cells (Wagner & Jaworski 1994) due, in part, to mRNA stabilization (Ellis & Wagner 1995). Chang et al. (1995) found that STC1 mRNA levels are also affected by extracellular Ca\(^{2+}\) concentration; the steady-state STC1 mRNA level in immortalized human fibroblasts was elevated almost 10-fold by a 2.5-fold increase in the Ca\(^{2+}\) concentration of the culture medium. It is unknown whether Ca\(^{2+}\) receptors are involved in this response.

The active metabolite of vitamin D\(_3\) has also been shown to affect STC1 expression. Treatment of female rats with calcitriol increased STC1 mRNA levels in the kidney more than 3-fold (Honda et al. 1999). This increase, however, may be due to vitamin D\(_3\)-induced hypercalcemia that has been observed to increase STC levels in catfish and male tilapia (Srivastav SP et al. 1985, Srivastav AK et al. 1998). Regulation of STC1 expression was tissue-specific; calcitriol treatment caused a marked increase of STC1 mRNA levels in the kidney, but levels in the ovary were unaffected.

A study by Sheikh-Hamad et al. (2000) on STC1 mRNA levels has shown a possible dual regulatory effect of hypercalcemia and extracellular Ca\(^{2+}\). In the canine renal cell line, MDCK, STC1 mRNA was induced 8-fold by growth in hypertonic medium. This induction of STC1 mRNA by hypercalcemia was dependent upon an extracellular Ca\(^{2+}\) concentration greater than 0.1 mM, confirming a role for extracellular Ca\(^{2+}\) in STC1 regulation.

Utilizing differential display analysis of mRNA from human umbilical vein endothelial cells untreated, or treated with lysophosphatidylcholine (lysoPC), a component of oxidized lipoproteins which is present in atherosclerotic lesions and has proatherogenic properties, Sato et al. (1998) identified 12 lysoPC-upregulated genes. STC1 was one of these genes and its expression was shown to be transitory with the maximum response seen after 6 h of lysoPC treatment. A significant increase in STC1 mRNA level was also observed by Kahn et al. (2000), who examined the endothelial differentiation of human umbilical vein cells in order to identify genes involved in angiogenesis. Furthermore, during capillary morphogenesis in 3-dimensional collagen matrices, STC1 was one of the genes that were most highly induced at 8, 24 and 48 h (Bell et al. 2001). Expression of STC1 was also found to be induced in response to hypoxia (Lal et al. 2001). Iyer et al. (1999), on the other hand, utilized a cDNA microarray to determine the temporal program of gene expression in serum-stimulated human fibroblasts, which are often used to investigate wound repair, and demonstrated induction of STC1 2 h after stimulation. Together the results suggest that STC1 may play a role in the pathogenesis of atherosclerosis, in the response to hypoxia, in wound repair and in angiogenesis.

Glucocorticoids were found to downregulate STC1 mRNA levels in the mouse corticotrope line, AIT-20, and the human fibrosarcoma line, HT1080 (Groves et al. 2001). It was postulated that the repression could be due to glucocorticoid-induced synthesis of proteins that interact directly or indirectly with STC1 mRNA to decrease its stability.
However, an alternative or additional explanation may be provided by the imperfect response element for the androgen/glucocorticoid/progesterone/mineralocorticoid receptor sub-family in the 5′ region of the STC1 gene (Bouras et al. 2002).

Interestingly, the downregulation of STC1 expression by glucocorticoids was antagonized by cAMP signaling (Groves et al. 2001). As mentioned above, cAMP and protein kinase A may also be involved in hCG-mediated stimulation of STC1 production in theca-interstitial cells in the ovary (Paciga et al. 2002).

Taken together, the available data indicate that expression of STC1 is controlled in a tissue-specific manner by a variety of stimuli, and most likely by a variety of signaling pathways.

**Evidence for an STC1 receptor**

Saturable, displaceable, high-affinity (0.25–0.8 nM) STC1 binding activity has been detected in the plasma membrane and inner mitochondrial matrix of liver and kidney (McCudden et al. 2002). The putative membrane receptor presumably facilitates translocation of STC1 to the mitochondria, and may also be coupled to a signal transduction mechanism. It is not clear at this stage whether the membrane and mitochondrial binding activities are the same. Mitochondrial localization is consistent with evidence that STC1 has a concentration-dependent stimulatory effect on mitochondrial electron transfer (McCudden et al. 2002), and the increased mitochondrial size in STC1-transgenic mice (Filvaroff et al. 2002).

**STC2 mRNA and protein expression**

Like STC1, human STC2 is also widely expressed in tissues including kidney, heart, pancreas and spleen. STC2 is expressed predominantly as a 2 kb transcript, with a rarer 4.4 kb transcript seen in some tissues (Chang & Reddel 1998, DiMattia et al. 1998, Ishibashi et al. 1998). STC2 protein was found in pancreatic α-cells (Moore et al. 1999), suggesting a possible role for STC2 in glucose homeostasis.

**Biological actions of STC2**

Using culture medium from STC2-transfected CHO cells, Ishibashi et al. (1998) examined the effect of STC2 on the promoter activity of a renal type II Na-P, cotransporter from the apical membrane of kidney proximal tubules. There was a significant reduction in promoter activity, suggesting that STC2 inhibited transcription of the cotransporter gene. Decreased P, uptake was observed in opossum kidney cells that were incubated in STC2-transfected CHO cell-conditioned medium for 2 days. This ability of STC2 to decrease P, uptake suggests that some STC2 effects may oppose those of STC1. A difference in the regulation of these two genes was observed by Honda et al. (1999), who showed a 20% reduction in STC2 mRNA levels coupled with a 3-fold increase in STC1 mRNA levels in the kidneys of calcitriol-treated rats.

In support of the concept that STC2 and STC1 may have different effects in some tissues, STC2 expression was found to be downregulated while STC1 was upregulated during neuronal differentiation of neuroblastoma cells (Wong et al. 2002). STC2 was unable to displace STC1 from its putative receptor, suggesting that STC2 has a separate receptor (McCudden et al. 2002).

**The secretion and phosphorylation of STC1 and STC2**

Additional evidence for differences between STC1 and STC2 has been obtained by comparing cellular lysates and conditioned medium from a human fibrosarcoma cell line, HT1080, which were immunoprecipitated with either STC1 or STC2 antibodies (Jellinek et al. 2000). As well as providing the first direct evidence for the secretion of STC1 and STC2 from human cells, this demonstrated that STC1 and STC2 are under different regulatory control. STC1 appeared to be sequestered intracellularly, possibly awaiting an external stimulus for secretion. STC2, on the other hand, was undetected in cellular lysates but present in conditioned medium, possibly indicating that the processing and secretion of STC2 is constitutive. Thus distinct processes regulate STC1 and STC2 secretion from the same cell, possibly reflecting different biological roles for each protein.

In addition, this study demonstrated that the mammalian STCs are secreted as phosphoproteins, most likely due to the action of ecto-kinases (Jellinek et al. 2000). The implications of this phosphorylation are unknown, but judging by the role of phosphorylation in regulating other secreted phosphoproteins (Saccuzzo et al. 1986, Feige et al. 1989, Wicks & Brooks 1995), it may be an important means of regulating structure, half-life, and/or biological activity of STC1 and STC2.

**Potential roles of STC1 and STC2 in cancer**

STC1 was originally cloned as part of a search for cancer-related genes (Chang et al. 1995). Once the full sequence was known, a cDNA fragment (designated S1) that had previously been found to be downregulated in 3 of 14 breast cancers (Liang et al. 1992) was identified in retrospect as a portion of the 3′ UTR of STC1. There is now increasing evidence of a role of the STCs in cancer, including breast cancer (Table 1).

STC1 is differentially expressed in a number of cancers compared with the relevant normal tissues.
of the genes showed and examined the expression of 12550 genes. About 0.4% positive breast cancer cell line, after exposure to estrogen, gen-responsive breast cancer cells, including the MCF-7 ER-(2000) performed serial analysis of gene expression on estrogen with the normal cells (Ismail 2002). STC1 was found to be downregulated 7-fold in ovarian cancer cells compared from which most ovarian tumors arise. STC1 was also found to be expressed in colon tumors at levels that were at least 10-fold higher than in normal mucosa (microarray); STC1 expression predominantly in vasculature (ISH)

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<td>STC1 expression increased (microarray); cell type not identified</td>
<td>Okabe et al. (2001)</td>
</tr>
<tr>
<td>Colon</td>
<td>STC1 expression increased (RT-PCR); cell type not identified</td>
<td>Fujiwara et al. (2000)</td>
</tr>
<tr>
<td>Ovarian</td>
<td>STC1 expression increased (IHC)</td>
<td>Watanabe et al. (2002)</td>
</tr>
<tr>
<td>MEN2B medullary thyroid cancer</td>
<td>STC1 downregulated (cDNA-RDA; Northern blot)</td>
<td>Ismail et al. (2000)</td>
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ISH, in situ hybridization; IHC, immunohistochemistry; RT-PCR, reverse-transcriptase polymerase chain reaction; cDNA-RDA, cDNA representational difference analysis.

Semiquantitative RT-PCR showed enhanced STC1 mRNA expression in hepatocellular and colorectal carcinomas compared with cancer-free tissues (Fujiwara et al. 2000). STC1 mRNA could be detected in the blood of 8 of 11 patients having surgery for hepatocellular carcinoma, in contrast to 0 of 31 normal donor blood samples, leading to the suggestion that STC1 mRNA might be a useful molecular marker for detection of tumor cells in blood (Fujiwara et al. 2000).

In a cDNA microarray study of 23 040 genes in 20 primary human hepatocellular carcinomas and their corresponding non-cancerous tissues, STC1 was among 165 genes found to be upregulated in 75% or more tumor samples (Okabe et al. 2001). STC1 was also found to be expressed in colon tumors at levels that were at least 10-fold higher than in normal mucosa, due primarily to expression of STC1 in the tumor vasculature (Gerritsen et al. 2002). A high level of STC1 expression was observed in medullary thyroid cancers from multiple endocrine neoplasia (MEN) 2B syndrome patients, and STC1 expression was found to be strongly induced by the RET-MEN2B mutant protein (Watanabe et al. 2002). cDNA representational difference analysis was used to compare expressed genes in human ovarian tumor cells and primary cultures of the ovarian surface epithelium from which most ovarian tumors arise. STC1 was found to be downregulated 7-fold in ovarian cancer cells compared with the normal cells (Ismail et al. 2000).

To identify estrogen-regulated genes, Charpentier et al. (2000) performed serial analysis of gene expression on estrogen-responsive breast cancer cell lines, including the MCF-7 ER-positive breast cancer cell line, after exposure to estrogen, and examined the expression of 12 550 genes. About 0.4% of the genes showed ≥ 3-fold increase, and these included STC2 (which showed a 10-fold upregulation). The level of STC1, however, was not affected. These results, when combined with other studies demonstrating STC2 expression in bone, suggest that STC2 may provide a link between estrogen and bone remodeling. In addition, the possibility that STC2 may be a useful breast cancer biomarker warrants further investigation (Charpentier et al. 2000).

In order to identify differences in gene expression between hormone-responsive and -unresponsive breast cancers, Bouras et al. (2002) used an oligonucleotide array corresponding to approximately 10 000 known genes and 25 000 EST clusters to identify genes induced by estrogen and repressed by the anti-estrogen ICI 182780. A highly significant correlation between the mRNA levels of STC2 and ER was found in a panel of 13 ER-positive and 12 ER-negative primary breast carcinomas. This result was corroborated by in situ hybridization and immunohistochemistry of a tissue microarray representing 236 breast carcinomas, which showed a significant correlation between STC2 mRNA and protein expression and ER status of the tumors (Fig. 3). STC1 expression was also examined and was found to correlate with the ER status of breast cancers. STC1 and STC2 mRNA levels both correlated with the protein expression of the ER target gene, progesterone receptor, and STC2 protein levels correlated with the expression of another ER target gene, pS2. Analysis of the STC2 5’ upstream sequence identified potential ER/Sp1-binding elements comparable with estrogen-responsive elements in many ER target genes. Although there was a strong correlation between expression of ER and both STC1 and STC2, the STCs were expressed only in a subset of ER-positive tumors. The authors suggested that STC1 and STC2 expression levels may provide clinically useful information beyond that provided by the ER status (Bouras et al. 2002). Interestingly, it was recently reported that expression levels of 21 genes, including STC2, in a panel of 301 breast cancers...
Figure 3 STC1 and STC2 mRNA and protein expression in ER-positive (tumors 1 and 2) and ER-negative (tumor 3) breast carcinoma sections. Immunohistochemical detection of ER protein is shown in (A, D and G). STC1 is shown for tumor 1 and STC2 for tumors 2 and 3: mRNA (B, E and H) and protein (C, F and I). In tumor 3, the STC2 staining is also representative of STC1 expression. From Bouras et al. (2002), with kind permission of the publisher.

had a greater prognostic significance than ER status and clinopathological parameters such as tumor size, lymph node metastases and histological grade (Iwao et al. 2002).

It was subsequently found that STC1 is induced by the tumor suppressor protein, BRCA1 (Welch et al. 2002). Germ-line mutations in the BRCA1 gene predispose to breast and ovarian cancer (Miki et al. 1994), and expression of BRCA1 is often reduced in sporadic breast cancers (Thompson et al. 1995). BRCA1 is found in multiprotein complexes involved in DNA repair and transcription (reviewed in Venkitaraman 2002). In a study designed to identify transcriptional targets of BRCA1, the neuronal lineage cell line HEK293 was transfected with an inducible BRCA1 expression plasmid, and oligonucleotide microarrays were used to compare gene expression in the presence and absence of upregulated BRCA1 levels (Welch et al. 2002). Consistent with previous studies showing that increased expression of BRCA1 represses ER-mediated transcription (Fan et al. 1999, Zheng et al. 2001), possibly via a direct association with ER-α (Fan et al. 2001), BRCA1 downregulated the ER-responsive genes, cyclin D1 and myc. On the other hand, BRCA1 upregulated STC1, as well as ID4, the JAK1 and STAT1 signaling molecules, and a subunit of the differentiation-associated laminin-5 protein. Of the genes studied, the expression of STC1 correlated most strongly with upregulated BRCA1 expression. BRCA1 and STC1 were found to be expressed in normal breast ductal epithelium and normal ovarian epithelium, and their expression was highly correlated in breast tumors, but not in ovarian tumors. The authors therefore suggested that loss of STC1 expression may be a marker of early breast tumorigenesis (Welch et al. 2002). In view of the evidence that (i) BRCA1 represses ER-mediated transcription and upregulates STC1 levels, (ii) estrogen strongly induces STC2 expression, and (iii) STC1 and STC2 are both associated with a subset of ER-positive tumors, expression of the STCs may be a
clinically useful marker of the interplay between BRCA1 and ER in breast cancers.

**Comments and perspectives on STC1, STC2 and cancer**

Although the data indicate that STC1 is commonly differentially expressed in tumors and corresponding normal tissues (Table 1), the significance of this observation awaits clarification. In particular, it needs to be determined whether the tumor cells are responsible for the differential expression. In the case of colon cancer, it seems that the increased expression may be contributed primarily by the tumor vasculature (Gerritsen et al. 2002). As discussed above, STC1 is highly expressed during angiogenesis.

The mechanisms of action of STC1 and STC2 also require clarification. On the basis of the anti-hypercalcemic role of STC in fish, it is tempting to conclude that their function is related to control of Ca²⁺. The expression of these proteins in mineralizing bone and in tissues involved in Ca²⁺ and P excretion or resorption, such as the kidney and breast, is consistent with such a function. The identification of regulatory molecules that control P levels would be of particular interest, because the regulation of P₇, homeostasis is still poorly understood, as are phosphate wasting disorders such as oncogenic osteomalacia (Strewler 2001). STC1 was excluded as the humoral factor responsible for the oncogenic osteomalacia caused by a hemangiopericytoma (Nelson et al. 1996), and if STC1 and STC2 have a role in oncogenic osteomalacia it would most likely be at the local tissue level. The existence of signal peptides in both STC1 and STC2, and the observation that STC1 mRNA and protein do not always colocalize (for example in the kidney, ovary and early post-implantation uterus) is consistent with extracellular secretion and local uptake. If it is correct that the roles of STC1 and STC2 are related in some way to control of Ca²⁺ and P levels at the local or tissue levels, the effects of these ions on cellular function are so pleiotropic that there is a very wide range of possibilities regarding the exact nature of their function in normal cells and in cancer.

It would be premature to rule out an endocrine role for STC1 and STC2, however, even though there is little evidence that they normally circulate in significant quantities, except STC1 during gestation. The finding of large STC1 forms in the ovary (Paciga et al. 2002) suggests that there might be a specific endocrine version of STC1. STC1 overexpression increases metabolic rate (Filvaroff et al. 2002), so it will be interesting to determine whether circulating STC1 contributes to malignant cachexia.

The effects of STC1 on metabolic rate and mitochondria raise a question regarding the possible relationship of STC1 expression to the Warburg effect. This is the reprogramming of tumor metabolism from oxidative to glycolytic metabolism, one of the most universal characteristics of solid tumors. Expression of genes encoding glucose transporters and glycolytic enzymes in tumor cells is increased by the transcription factors c-Myc and hypoxia-inducible factor (HIF)-1 (reviewed in Maxwell et al. 2001). Lactate and pyruvate are also able to upregulate HIF-1, and it has therefore been suggested that the cancer-associated increase in glycolysis may be a feedforward mechanism to maintain the expression of genes turned on by HIF-1 (Lu et al. 2002). STC1 expression increased electron transfer (McCudden et al. 2002) and increased mitochondrial size (Filvaroff et al. 2002), which would tend to reduce the pool of lactate and pyruvate, so STC1 might modulate the putative HIF-1/pyruvate positive feedback loop. Decreased STC1 expression in tumors such as ER-negative breast cancer and ovarian cancer might potentiate the Warburg effect.

STC1 is one of a very small number of regulatory factors that are thought to have direct effects on mitochondrial electron transfer (the others being nitric oxide and thyroid hormone), one of only two polypeptide hormones targeted to the mitochondrion (the other being transforming growth factor β-1), and the only polypeptide hormone thought to have a mitochondrial receptor (McCudden et al. 2002). Interestingly, comparison of RNA expression patterns before and after immortalization of SV40-transformed cells identified not only STC1 (Chang et al. 1995), but also altered transcription of mitochondrial DNA (Duncan et al. 2000).

Experimental data also suggest that STC1 may play a role in other cellular processes. Based upon the increased resistance of STC1-transfected neural cells to hypoxia or ischemia, Zhang et al. (1998) hypothesized that STC1 may protect cellular integrity. Although hypoxic cell death has generally been considered to be manifested as necrosis, recent biochemical evidence suggests that hypoxia may induce apoptosis (Banasiaik et al. 2000). Could STC1 contribute to cellular survival by inhibiting some aspect of the apoptotic pathway? The high level of STC1 in terminally differentiated cells with extended survival, e.g. neurons (Zhang et al. 1998) and megakaryocytes (Serlachius et al. 2002), is also consistent with a prosurvival function of STC1.

Sheikh-Hamad et al. (2000) postulated that STC1 might play a cytoprotective role in MDCK cells by inhibiting Ca²⁺ entry under hypertonic conditions. A hyperosmolar environment may directly affect cell volume, and there is evidence that loss of cell volume may be a defining early characteristic of apoptosis. Moreover, the regulation of cell volume could be mediated by intracellular ion levels, thus supporting a relationship between changes in ion flux and apoptosis. Ca²⁺ plays a key role in this process as evidenced by the influx of Ca²⁺ into the cell with the onset of apoptosis, as well as by the ability of Ca²⁺ channel blockers to inhibit apoptosis (Manion et al. 2000). Whether STC1 has a role in this remains to be seen.

A number of other observations about the STCs would also be consistent with a paracrine and/or autocrine...
prosurvival role for these proteins. At the cellular level, prolif-eration requires signaling via both pro-proliferative and anti-apoptotic/prosurvival pathways. STC1 is induced rapidly by serum-stimulation of fibroblasts and during angiogenesis, so it seems possible that STC1 has a prosurvival function at the tissue level during wound healing. The pattern of STC1 expression in the ovary may be related to a paracrine prosur-vival effect exerted by secondary interstitial and theca interna cells on oocytes and the corpus luteum, and circulating STC1 during gestation may exert a prosurvival effect, for example, on the rapidly proliferating cells of the mammary gland. Even the expression pattern in the kidney might be explained as a prosurvival signal to cells affected by hyperosmotic stresses.

How does secreted STC1 or STC2 exert its putative paracrine effects? The presence of high-affinity binding sites in plasma membranes and mitochondria (McCudden et al. 2002) would explain the selective uptake of these proteins by specific cells within tissues. The receptor molecule(s) await identification. Even though there is evidence suggesting that the mammalian STCs may have opposing effects in some contexts, and that STC2 is unable to displace STC1 from its receptor, given their level of homology and the fact that many different cell types produce both proteins, the possibility of heterodimer formation needs to be investigated. Furthermore, the effects of post-translational modifications, including glycosylation and phosphorylation, remain to be elucidated.

It may be instructive to note some potential parallels and contrasts between the STCs and PTH-related protein (PTHRp). The latter was originally discovered as a tumor hormone and a possible extracorpuscular distribution. It has been implicated in the regulation of a variety of biological processes such as cell growth and differentiation, the regulation of smooth muscle tone and the facilitation of placental Ca\textsuperscript{2+} transport (Philbrick et al. 1996). Unlike PTH, which is produced by the parathyroid glands, and circulates as a classic peptide hormone that regulates systemic Ca\textsuperscript{2+} metabolism, PTHrP is produced by many normal cell types during development and adult life and exerts its actions locally. It has been implicated in the regulation of a variety of biological processes such as cell growth and differentiation, the regulation of smooth muscle tone and the facilitation of placental Ca\textsuperscript{2+} transport (Philbrick et al. 1996). Disruption of PTHrP and PTH/PTHrP receptor genes affected chondrocyte differentiation during endochondral bone development leading to neonatal death of the knockout mice (Karaplis et al. 1994, Lanske et al. 1996, Vortkamp et al. 1996). A recent study using PTHrP-knockout mice that were rescued from neonatal death by targeted transgenic expression of PTHrP in chondrocytes suggested that it plays a critical role in the epithelial–mesenchymal communications that guide the initial round of branching morphogenesis that occurs during mammary gland development (Wysolmerski et al. 1998). PTHrP expression in breast cancers correlates with ER status (Henderson et al. 2001), and is a favorable prognostic indicator. It is of considerable interest that PTHrP seems to have a role both in Ca\textsuperscript{2+} mobilization and in development of the breast, the organ responsible for transfer of maternal Ca\textsuperscript{2+} to the neonate.

In addition to the questions raised above, there is clearly much more to be learnt about STC1 and STC2. Studies addressing the prognostic significance of STC1 and STC2 expression in breast cancer are needed, as well as studies of these proteins in other cancers. Cancers of tissues in which these proteins are normally expressed abundantly, including ovary, prostate, thyroid and bone, would be of particular interest. In view of the accumulating evidence that STC1 is involved in mesenchymal–epithelial signaling, it will also be interesting to determine whether expression of STC1 and/or STC2 by normal tissues contributes to the characteristic tissue distribution of metastases from specific types of carcinomas. Many of the existing studies in normal tissues have focused on cellular localization, and studies of function are mostly at a preliminary stage. It will be of interest to determine whether the STCs, like PTHrP, have a role in normal mammary development. Transgenic mice that overexpress STC1 or STC2 in various tissues, and also animals that have been rendered null for these genes by gene targeting, should be informative regarding normal functions of STC1 and STC2 and their roles in carcinogenesis.

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