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_i) 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 (Welsh *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 (Welsh *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 teleostean 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 Ishibashi *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 (Lafeber *et al.* 1988b). Disulfide linkages in chum salmon STC have been defined (Hulova & Kawachi 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

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 eclustal program (<http://www.angis.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.

Figure 2 Genomic structure of fish STC, and human STC1 and STC2. Exons are represented by boxes, introns by the intervening lines between exons, protein coding region by shading, and UTRs by the open portion of the boxes. Size of exons and introns is shown in kb; number of amino acid residues (aa) is shown for each exon.

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 α -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, Wong *et al.* 1998). In human kidney, De Niu *et al.* (1998) localized STC1 to principal and α -intercalated cells in the distal half of the nephron, a distribution that is in good agreement with what has been reported in rat and mouse kidney (Haddad *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 ~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, than age-matched 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 P_i homeostasis. Recombinant STC1 inhibited uptake of Ca^{2+} through the gills when injected into goldfish, and stimulated renal P_i resorption and reduced renal P_i 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 P_i 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^+/P_i 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 P_i 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 P_i 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 I 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 P_i levels indicating that overexpression of STC1 perturbs mineral homeostasis. This effect on serum P_i is consistent with the evidence that STC1 can stimulate P_i 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 degranulation 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 hypertonicity 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 hypertonicity 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, AtT-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_i 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_i 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_i 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.

Table 1 Expression of STC1 and STC2 in human tumors

Tumor type	Expression data (detection method)	Reference
Breast adenocarcinomas and other tumors	High level expression of STC1 mRNA in tumor vasculature (ISH)	Kahn <i>et al.</i> (2000)
Breast	STC1 and STC2 expressed in a subset of ER-positive tumors (ISH and IHC)	Bouras <i>et al.</i> (2002)
Breast and ovary	STC1 expression decreased in breast and ovarian cancer; STC1 expression highly correlated with BRCA1 expression in breast but not ovarian cancer (IHC)	Welsh <i>et al.</i> (2002)
Colon	STC1 expression increased 10-fold in tumors compared with normal mucosa (microarray); STC1 expression predominantly in vasculature (ISH)	Gerritsen <i>et al.</i> (2002)
Hepatocellular	STC1 expression increased (microarray); cell type not identified	Okabe <i>et al.</i> (2001)
Hepatocellular and colon	STC1 expression increased (RT-PCR); cell type not identified	Fujiwara <i>et al.</i> (2000)
MEN2B medullary thyroid cancer	STC1 expression increased (IHC)	Watanabe <i>et al.</i> (2002)
Ovarian	STC1 downregulated (cDNA-RDA; Northern blot)	Ismail <i>et al.</i> (2000)

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 cells, 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 in MCF-7 cells. STC2 was one of the genes that met these criteria, being upregulated 3-fold by estrogen at 3 h and downregulated 3-fold by 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 clinicopathological 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 (Welsh *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 (Welsh *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 down-regulated 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 (Welsh *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 functions may be somehow related to control of Ca^{2+} and P_i . The expression of these proteins in mineralizing bone and in tissues involved in Ca^{2+} and P_i excretion or resorption, such as the kidney and breast, is consistent with such a function. The identification of regulatory molecules that control P_i levels would be of particular interest, because the regulation of P_i 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 Ca^{2+} and P_i 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 (Banasiak *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 Ca^{2+} 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. Ca^{2+} plays a key role in this process as evidenced by the influx of Ca^{2+} into the cell with the onset of apoptosis, as well as by the ability of Ca^{2+} 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, proliferation 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 prosurvival 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 product that causes humoral hypercalcemia of malignancy (Philbrick *et al.* 1996, Martin & Moseley 2000). Unlike PTH, which is produced by the parathyroid glands, and circulates as a classic peptide hormone that regulates systemic Ca^{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^{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^{2+} mobilization and in development of the breast, the organ responsible for transfer of maternal Ca^{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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References

- Aida K, Nishioka RS & Bern HA 1980 Degranulation of the Stannius corpuscles of coho salmon (*Oncorhynchus kisutch*) in response to ionic changes in vitro. *General and Comparative Endocrinology* **41** 305–313.
- Amemiya Y, Marra LE, Reyhani N & Youson JH 2002 Stanniocalcin from an ancient teleost: a monomeric form of the hormone and a possible extracorporeal distribution. *Molecular and Cellular Endocrinology* **188** 141–150.
- Banasik KJ, Xia Y & Haddad GG 2000 Mechanisms underlying hypoxia-induced neuronal apoptosis. *Progress in Neurobiology* **62** 215–249.
- Bell SE, Mavila A, Salazar R, Bayless KJ, Kanagala S, Maxwell SA & Davis GE 2001 Differential gene expression during capillary morphogenesis in 3D collagen matrices: regulated expression of genes involved in basement membrane matrix assembly, cell cycle progression, cellular differentiation and G-protein signaling. *Journal of Cell Science* **114** 2755–2773.
- Bouras T, Southey MC, Chang AC, Reddel RR, Willhite D, Glynne R, Henderson MA, Armes JE & Venter DJ 2002 Stanniocalcin 2 is an estrogen-responsive gene coexpressed with

- the estrogen receptor in human breast cancer. *Cancer Research* **62** 1289–1295.
- Butkus A, Roche PJ, Fernley RT, Haralambidis J, Penschow JD, Rya GB, Trahair JF, Tregear GW & Coghlan JP 1987 Purification and cloning of a corpuscles of Stannius protein from *Anguilla australis*. *Molecular and Cellular Endocrinology* **54** 123–133.
- Chang ACM & Reddel RR 1998 Identification of a second stanniocalcin cDNA in mouse and human: stanniocalcin 2. *Molecular and Cellular Endocrinology* **141** 95–99.
- Chang ACM, Janosi J, Hulsbeek M, De Jong D, Jeffrey KJ, Noble JR & Reddel RR 1995 A novel human cDNA highly homologous to the fish hormone stanniocalcin. *Molecular and Cellular Endocrinology* **112** 241–247.
- Chang ACM, Jeffrey KJ, Tokutake Y, Shimamoto A, Neumann AA, Dunham MA, Cha J, Sugawara M, Furuichi Y & Reddel RR 1998 Human stanniocalcin (STC): genomic structure, chromosomal localization, and the presence of CAG trinucleotide repeats. *Genomics* **47** 393–398.
- Charpentier AH, Bednarek AK, Daniel RL, Hawkins KA, Laffin KJ, Gaddis S, MacLeod MC & Aldaz CM 2000 Effects of estrogen on global gene expression: identification of novel targets of estrogen action. *Cancer Research* **60** 5977–5983.
- De Niu P, Olsen HS, Gentz R & Wagner GF 1998 Immunolocalization of stanniocalcin in human kidney. *Molecular and Cellular Endocrinology* **137** 155–159.
- De Niu P, Radman DP, Jaworski EM, Deol H, Gentz R, Su J, Olsen HS & Wagner GF 2000 Development of a human stanniocalcin radioimmunoassay: serum and tissue hormone levels and pharmacokinetics in the rat. *Molecular and Cellular Endocrinology* **162** 131–144.
- Deol HK, Varghese R, Wagner GF & DiMattia GE 2000 Dynamic regulation of mouse ovarian stanniocalcin expression during gestation and lactation. *Endocrinology* **141** 3412–3421.
- DiMattia GE, Varghese R & Wagner GF 1998 Molecular cloning and characterization of stanniocalcin-related protein. *Molecular and Cellular Endocrinology* **146** 137–140.
- Duncan EL, Perrem K & Reddel RR 2000 Identification of a novel human mitochondrial D-loop RNA species which exhibits upregulated expression following cellular immortalization. *Biochemical and Biophysical Research Communications* **276** 439–446.
- Ellis TJ & Wagner GF 1995 Post-transcriptional regulation of the stanniocalcin gene by calcium. *Journal of Biological Chemistry* **270** 1960–1965.
- Fan S, Wang J-A, Yuan R, Ma Y, Meng Q, Erdos MR, Pestell RG, Yuan F, Auborn KJ, Goldberg ID & Rosen EM 1999 BRCA1 inhibition of estrogen receptor signaling in transfected cells. *Science* **284** 1354–1356.
- Fan S, Ma YX, Wang C, Yuan RQ, Meng Q, Wang JA, Erdos M, Goldberg ID, Webb P, Kushner PJ, Pestell RG & Rosen EM 2001 Role of direct interaction in BRCA1 inhibition of estrogen receptor activity. *Oncogene* **20** 77–87.
- Feige JJ, Bradley JD, Fryburg K, Farris J, Cousens LC, Barr PJ & Baird A 1989 Differential effects of heparin, fibronectin, and laminin on the phosphorylation of basic fibroblast growth factor by protein kinase C and the catalytic subunit of protein kinase A. *Journal of Cell Biology* **109** 3105–3114.
- Filvaroff EH, Guillet S, Zlot C, Bao M, Ingle G, Steinmetz H, Hoeffel J, Bunting S, Ross J, Carano RA, Powell-Braxton L, Wagner GF, Eckert R, Gerritsen ME & French DM 2002 Stanniocalcin 1 alters muscle and bone structure and function in transgenic mice. *Endocrinology* **143** 3681–3690.
- Fontaine M 1964 Corpuscles de Stannius et régulation ionique (Ca, K, Na) du milieu intérieur de l'anguille (*Anguilla anguilla* L.). *Comptes Rendus Hebdomadaire des Séances de l'Académie des Sciences D* **259** 875–878.
- Franzén AM, Zhang K, Westberg JA, Zhang W-M, Arola J, Olsen HS & Andersson LC 2000 Expression of stanniocalcin in the epithelium of human choroid plexus. *Brain Research* **887** 440–443.
- Fraser RA, Kaneko T, Pang PK & Harvey S 1991 Hypo- and hypercalcemic peptides in fish pituitary glands. *American Journal of Physiology* **260** R622–R626.
- Fujiwara Y, Sugita Y, Nakamori S, Miyamoto A, Shiozaki K, Nagano H, Sakon M & Monden M 2000 Assessment of stanniocalcin-1 mRNA as a molecular marker for micrometastases of various human cancers. *International Journal of Oncology* **16** 799–804.
- Gerritsen ME, Soriano R, Yang S, Ingle G, Zlot C, Toy K, Winer J, Draksharapu A, Peale F, Wu TD & Williams PM 2002 *In silico* data filtering to identify new angiogenesis targets from a large in vitro gene profiling data set. *Physiological Genomics* **10** 13–20.
- Groves TC, Wagner GF & DiMattia GE 2001 cAMP signaling can antagonize potent glucocorticoid post-transcriptional inhibition of stanniocalcin gene expression. *Journal of Endocrinology* **171** 499–516.
- Haddad M, Roder S, Olsen HS & Wagner GF 1996 Immunocytochemical localization of stanniocalcin cells in the rat kidney. *Endocrinology* **137** 2113–2117.
- Hanssen RGJM, Labeber FPJG, Flik G & Wendelaar Bonga SE 1989 Ionic and total calcium levels in the blood of the European eel (*Anguilla anguilla*): effects of stannectomy and hypocalcin replacement therapy. *Journal of Experimental Biology* **141** 177–186.
- Hanssen RGJM, Aarden EM, Van der Venne WPHG, Pang PKT & Wendelaar Bonga SE 1991 Regulation of secretion of the teleost fish hormone stanniocalcin: effects of extracellular calcium. *General and Comparative Endocrinology* **84** 155–163.
- Henderson M, Danks J, Moseley J, Slavin J, Harris T, McKinlay M, Hopper J & Martin T 2001 Parathyroid hormone-related protein production by breast cancers, improved survival, and reduced bone metastases. *Journal of the National Cancer Institute* **93** 234–237.
- Honda S, Kashiwagi M, Ookata K, Tojo A & Hirose S 1999 Regulation by $1\alpha,25$ -dihydroxyvitamin D_3 of expression of stanniocalcin messages in the rat kidney and ovary. *FEBS Letters* **459** 119–122.
- Hulova I & Kawauchi H 1999 Assignment of disulfide linkages in chum salmon stanniocalcin. *Biochemical and Biophysical Research Communications* **257** 295–299.
- Ishibashi K & Imai M 2002 Prospect of a stanniocalcin endocrine/paracrine system in mammals. *American Journal of Physiology. Renal Physiology* **282** F367–F375.
- Ishibashi K, Miyamoto K, Taketani Y, Morita K, Takeda E, Sasaki S & Imai I 1998 Molecular cloning of a second human stanniocalcin homologue (STC2). *Biochemical and Biophysical Research Communications* **250** 252–258.
- Ismail RS, Baldwin RL, Fang J, Browning D, Karlan BY, Gasson JC & Chang DD 2000 Differential gene expression between normal and tumor-derived ovarian epithelial cells. *Cancer Research* **60** 6744–6749.

- Iwao K, Matoba R, Ueno N, Ando A, Miyoshi Y, Matsubara K, Noguchi S & Kato K 2002 Molecular classification of primary breast tumors possessing distinct prognostic properties. *Human Molecular Genetics* **11** 199–206.
- Iyer VR, Eisen MB, Ross DT, Schuler G, Moore T, Lee JCF, Trent JM, Staudt LM, Hudson J Jr, Boguski MS, Lashkari D, Shalon D, Botstein D & Brown PO 1999 The transcriptional program in the response of human fibroblasts to serum. *Science* **283** 83–87.
- Jellinek DA, Chang AC, Larsen MR, Wang X, Robinson PJ & Reddel RR 2000 Stanniocalcin 1 and 2 are secreted as phosphoproteins from human fibrosarcoma cells. *Biochemical Journal* **350** 453–461.
- Jiang W-Q, Chang ACM, Satoh M, Furuichi Y, Tam PPL & Reddel RR 2000 The distribution of stanniocalcin 1 protein in fetal mouse tissues suggests a role in bone and muscle development. *Journal of Endocrinology* **165** 457–466.
- Kahn J, Mehraban F, Ingle G, Xin X, Bryant JE, Vehar G, Schoenfeld J, Grimaldi CJ, Peale F, Draksharapu A, Lewin DA & Gerritsen ME 2000 Gene expression profiling in an *in vitro* model of angiogenesis. *American Journal of Pathology* **156** 1887–1900.
- Karaplis AC, Luz A, Glowacki J, Bronson RT, Tybulewicz VLJ, Kronenberg HM & Mulligan RC 1994 Lethal skeletal dysplasia from targeted disruption of the parathyroid hormone-related peptide gene. *Genes and Development* **8** 277–289.
- Lafeber FPJG & Perry SF 1988 Experimental hypercalcemia induces hypocalcin release and inhibits branchial Ca^{2+} influx in freshwater trout. *General and Comparative Endocrinology* **72** 136–143.
- Lafeber FPJG, Schaeffer HIMB, Herrmann-Erlee MPM & Wendelaar Bonga SE 1986 Parathyroid hormone-like effects of rainbow trout Stannius products on bone resorption of embryonic mouse calvaria *in vitro*. *Endocrinology* **119** 2249–2255.
- Lafeber FPJG, Flik G, Wendelaar Bonga SE & Perry SF 1988a Hypocalcin from Stannius corpuscles inhibits gill calcium uptake in trout. *American Journal of Pathology* **254** R891–R896.
- Lafeber FPJG, Hanssen RGJM, Choy YM, Flik G, Herrmann-Erlee MPM, Pang PKT & Wendelaar Bonga SE 1988b Identification of hypocalcin (teleocalcin) isolated from trout Stannius corpuscles. *General and Comparative Endocrinology* **69** 19–30.
- Lal A, Peters H, St Croix B, Haroon ZA, Dewhirst MW, Strausberg RL, Kaanders JHAM, van der Kogel AJ & Riggins GJ 2001 Transcriptional response to hypoxia in human tumors. *Journal of the National Cancer Institute* **93** 1337–1343.
- Lanske B, Karaplis AC, Lee K, Luz A, Vortkamp A, Pirro A, Karperien M, Defize LH, Ho C, Mulligan RC, Abou-Samra AB, Juppner H, Segre GV & Kronenberg HM 1996 PTH/PTHrP receptor in early development and Indian hedgehog-regulated bone growth. *Science* **273** 663–666.
- Liang P, Averboukh L, Keyomarsi K, Sager R & Pardee AB 1992 Differential display and cloning of messenger RNAs from human breast cancer versus mammary epithelial cells. *Cancer Research* **52** 6966–6968.
- Lopez E, Tisserand-Jochem E-M, Eyquem A, Milet C, Hillyard C, Lallier F, Vidal B & Mac Intyre I 1984 Immunocytochemical detection in eel corpuscles of Stannius of a mammalian parathyroid-like hormone. *General and Comparative Endocrinology* **53** 28–36.
- Lu H, Forbes RA & Verma A 2002 Hypoxia-inducible factor 1 activation by aerobic glycolysis implicates the Warburg effect in carcinogenesis. *Journal of Biological Chemistry* **277** 23111–23115.
- Lu M, Wagner GF & Renfro JL 1994 Stanniocalcin stimulates phosphate reabsorption by flounder renal proximal tubule in primary culture. *American Journal of Pathology* **267** R1356–R1362.
- Madsen KL, Tavernini MM, Yachimec C, Mendrick DL, Alfonso PJ, Buerger M, Olsen HS, Antonaccio MJ, Thomson ABR & Fedorak RN 1998 Stanniocalcin: a novel protein regulating calcium and phosphate transport across mammalian intestine. *American Journal of Pathology* **274** G96–G102.
- Manion MK, Su Z, Villain M & Blalock JE 2000 A new type of Ca^{2+} channel blocker that targets Ca^{2+} sensors and prevents Ca^{2+} -mediated apoptosis. *FASEB Journal* **14** 1297–1306.
- Martin TJ & Moseley JM 2000 Mechanisms in the skeletal complications of breast cancer. *Endocrine-Related Cancer* **7** 271–284.
- Maxwell PH, Pugh CW & Ratcliffe PJ 2001 Activation of the HIF pathway in cancer. *Current Opinion in Genetics and Development* **11** 293–299.
- McCudden CR, Kogon MR, DiMattia GE & Wagner GF 2001a Novel expression of the stanniocalcin gene in fish. *Journal of Endocrinology* **171** 33–44.
- McCudden CR, Tam WH & Wagner GF 2001b Ovarian stanniocalcin in trout is differentially glycosylated and preferentially expressed in early stage oocytes. *Biology of Reproduction* **65** 763–770.
- McCudden CR, James KA, Hasilo C & Wagner GF 2002 Characterization of mammalian stanniocalcin receptors: mitochondrial targeting of ligand and receptor for regulation of cellular metabolism. *Journal of Biological Chemistry* **277** 45249–45258.
- Miki Y, Swensen J, Shattuck-Eidens D, Futreal PA, Harshman K, Tavtigian S, Liu Q, Cochran C, Bennett LM, Ding W, Bell R, Rosenthal J, Hussey C, Tran T, McClure M, Frye C, Hattier T, Phelps R, Haugen-Strano A, Katcher H, Yakumo K, Gholami Z, Shaffer D, Stone S, Bayer S, Wray C, Bogden R, Dayananth P, Ward J, Tonin P, Narod S, Bristow PK, Norris FH, Helvering L, Morrison P, Rostek P, Lai M, Barrett JC, Lewis C, Neuhausen S, Cannon-Albright L, Goldgar D, Wiseman R, Kamb A & Skolnick MH 1994 A strong candidate for the breast and ovarian cancer susceptibility gene *BRCA1*. *Science* **266** 66–71.
- Moore EE, Kuestner RE, Conklin DC, Whitmore TE, Downey W, Biddle MM, Adams RL, Bell LA, Thompson DL, Wolf A, Chen L, Stamm MR, Grant FJ, Lok S, Ren H & De Jongh KS 1999 Stanniocalcin 2: characterization of the protein and its localization to human pancreatic alpha cells. *Hormone and Metabolism Research* **31** 406–414.
- Nelson AE, Namkung HJ, Patava J, Wilkinson MR, Chang ACM, Reddel RR, Robinson BG & Mason RS 1996 Characteristics of tumor cell bioactivity in oncogenic osteomalacia. *Molecular and Cellular Endocrinology* **124** 17–23.
- Okabe H, Satoh S, Kato T, Kitahara O, Yanagawa R, Yamaoka Y, Tsunoda T, Furukawa Y & Nakamura Y 2001 Genome-wide analysis of gene expression in human hepatocellular carcinomas using cDNA microarray: identification of genes involved in liver carcinogenesis and tumor progression. *Cancer Research* **61** 2129–2137.
- Olsen HS, Cepeda MA, Zhang QQ, Rosen CA, Vozzolo BL & Wagner GF 1996 Human stanniocalcin: a possible hormonal regulator of mineral metabolism. *PNAS* **93** 1792–1796.

- Paciga M, Watson AJ, DiMattia GE & Wagner GF 2002 Ovarian stanniocalcin is structurally unique in mammals and its production and release are regulated through the luteinizing hormone receptor. *Endocrinology* **143** 3925–3934.
- Philbrick WM, Wysolmerski JJ, Galbraith S, Holt E, Orloff JJ, Yang KH, Vasavada RC, Weir EC, Broadus AE & Stewart AF 1996 Defining the roles of parathyroid hormone-related protein in normal physiology. *Physiological Reviews* **76** 127–173.
- Radman DP, McCudden C, James K, Nemeth EM & Wagner GF 2002 Evidence for calcium-sensing receptor mediated stanniocalcin secretion in fish. *Molecular and Cellular Endocrinology* **186** 111–119.
- Reddy PS & Housman DE 1997 The complex pathology of trinucleotide repeats. *Current Opinion in Cell Biology* **9** 364–372.
- Saccuzzo JE, Krzesicki RF, Perini F & Ruddon RW 1986 Phosphorylation of the secreted, free alpha subunit of human chorionic gonadotropin. *PNAS* **83** 9493–9496.
- Sato N, Kokame K, Shimokado K, Kato H & Miyata T 1998 Changes of gene expression by lysophosphatidylcholine in vascular endothelial cells: 12 up-regulated distinct genes including 5 cell growth-related, 3 thrombosis-related, and 4 others. *Journal of Biochemistry* **123** 1119–1126.
- Serlachius M, Alitalo R, Olsen HS & Andersson LC 2002 Expression of stanniocalcin-1 in megakaryocytes and platelets. *British Journal of Haematology* **119** 359–363.
- Sheikh-Hamad D, Rouse D & Yang Y 2000 Regulation of stanniocalcin in MDCK cells by hypertonicity and extracellular calcium. *American Journal of Physiology* **278** F417–F424.
- Srivastav AK, Flik G & Wendelaar Bonga SE 1998 Plasma calcium and stanniocalcin levels of male tilapia, *Oreochromis mossambicus*, fed calcium-deficient food and treated with 1,25 dihydroxyvitamin D₃. *General and Comparative Endocrinology* **110** 290–294.
- Srivastav SP, Swarup K & Srivastav AK 1985 Structure and behaviour of Stannius corpuscles in relation to vitamin D₃ induced hypercalcemia in male *Clarias batrachus*. *Molecular and Cellular Biology* **31** 1–5.
- Stannius H 1839 Nebennieren bei knochenfischen. *Archiv für Anatomie und Physiologie* **6** 97–101.
- Stasko SE & Wagner GF 2001a Possible roles for stanniocalcin during early skeletal patterning and joint formation in the mouse. *Journal of Endocrinology* **171** 237–248.
- Stasko SE & Wagner GF 2001b Stanniocalcin gene expression during mouse urogenital development: a possible role in mesenchymal–epithelial signalling. *Development Dynamics* **220** 49–59.
- Stasko SE, DiMattia GE & Wagner GF 2001 Dynamic changes in stanniocalcin gene expression in the mouse uterus during early implantation. *Molecular and Cellular Biology* **174** 145–149.
- Strewler GJ 2001 FGF23, hypophosphatemia, and rickets: has phosphatonin been found? *PNAS* **98** 5945–5946.
- Sundell K, Björnsson BTH, Itoh H & Kawauchi H 1992 Chum salmon (*Oncorhynchus keta*) stanniocalcin inhibits *in vitro* calcium uptake in Atlantic cod (*Gadus morhua*). *Journal of Comparative Physiology B* **162** 489–495.
- Takagi Y, Nakamura Y & Yamada J 1985 Effects of the removal of the corpuscles of Stannius on the transport of calcium across the intestine of rainbow trout. *Zoological Sciences* **2** 523–530.
- Thompson ME, Jensen RA, Obermiller PS, Page DL & Holt JT 1995 Decreased expression of BRCA1 accelerates growth and is often present during sporadic breast cancer progression. *Nature Genetics* **9** 444–450.
- Varghese R, Wong CKC, Deol H, Wagner GF & DiMattia GE 1998 Comparative analysis of mammalian stanniocalcin genes. *Endocrinology* **139** 4714–4725.
- Varghese R, Gagliardi AD, Bialek PE, Yee SP, Wagner GF & DiMattia GE 2002 Overexpression of human stanniocalcin affects growth and reproduction in transgenic mice. *Endocrinology* **143** 868–876.
- Venkitaraman AR 2002 Cancer susceptibility and the functions of BRCA1 and BRCA2. *Cell* **108** 171–182.
- Vortkamp A, Lee K, Lanske B, Segre GV, Kronenberg HM & Tabin CJ 1996 Regulation of rate of cartilage differentiation by Indian hedgehog and PTH-related protein. *Science* **273** 613–622.
- Wagner GF & Jaworski E 1994 Calcium regulates stanniocalcin mRNA levels in primary cultured rainbow trout corpuscles of Stannius. *Molecular and Cellular Biology* **99** 315–322.
- Wagner GF, Fenwick JC, Park CM, Milliken C, Copp DH & Friesen HG 1988 Comparative biochemistry and physiology of teleocalcin from sockeye and coho salmon. *General and Comparative Endocrinology* **72** 237–246.
- Wagner GF, DiMattia GE, Davie JR, Copp DH & Friesen HG 1992 Molecular cloning and cDNA sequence analysis of coho salmon stanniocalcin. *Molecular and Cellular Biology* **90** 7–15.
- Wagner GF, Guiraudon CC, Milliken C & Copp DH 1995 Immunological and biological evidence for a stanniocalcin-like hormone in human kidney. *PNAS* **92** 1871–1875.
- Wagner GF, Vozzolo BL, Jaworski E, Haddad M, Kline RL, Olsen HS, Rosen CA, Davidson MB & Renfro JL 1997 Human stanniocalcin inhibits renal phosphate excretion in the rat. *Journal of Bone Mineral Research* **12** 165–171.
- Watanabe T, Ichihara M, Hashimoto M, Shimono K, Shimoyama Y, Nagasaka T, Murakumo Y, Murakami H, Sugiura H, Iwata H, Ishiguro N & Takahashi M 2002 Characterization of gene expression induced by RET with MEN2A or MEN2B mutation. *American Journal of Pathology* **161** 249–256.
- Welsh PL, Lee MK, Gonzalez-Hernandez RM, Black DJ, Mahadevappa M, Swisher EM, Warrington JA & King MC 2002 BRCA1 transcriptionally regulates genes involved in breast tumorigenesis. *PNAS* **99** 7560–7565.
- Wendelaar Bonga SE & Pang PKT 1991 Control of calcium regulating hormones in the vertebrates: parathyroid hormone, calcitonin, prolactin, and stanniocalcin. *International Review of Cytology* **128** 139–213.
- Wendelaar Bonga SE, Labefer FPJG, Flik G, Kaneko T & Pang PKT 1989 Immunocytochemical demonstration of a novel system of neuroendocrine peptidergic neurons in the pond snail *Lymnaea stagnalis*, with antisera to the teleostean hormone hypocalcin and mammalian parathyroid hormone. *General and Comparative Endocrinology* **75** 29–38.
- White KE, Biber J, Murer H & Econs MJ 1998 Chromosomal localization of two human genes involved in phosphate homeostasis: the type IIb sodium-phosphate cotransporter and stanniocalcin-2. *Somatic Cell and Molecular Genetics* **24** 357–362.
- Wicks JR & Brooks CL 1995 Biological activity of phosphorylated and dephosphorylated bovine prolactin. *Molecular and Cellular Biology* **112** 223–229.
- Wong CKC, Ho MA & Wagner GF 1998 The co-localization of stanniocalcin protein, mRNA and kidney cell markers in the rat kidney. *Journal of Endocrinology* **158** 183–189.

- Wong CKC, Yeung HY, Mak NK, DiMattia GE, Chan DK & Wagner GF 2002 Effects of dibutyryl cAMP on stanniocalcin and stanniocalcin-related protein mRNA expression in neuroblastoma cells. *Journal of Endocrinology* **173** 199–209.
- Worthington RA, Brown L, Jellinek D, Chang ACM, Reddel RR, Hambly BD & Barden JA 1999 Expression and localisation of stanniocalcin 1 in rat bladder, kidney and ovary. *Electrophoresis* **20** 2071–2076.
- Wysolmerski JJ, Philbrick WM, Dunbar ME, Lanske B, Kronenberg H & Broadus AE 1998 Rescue of the parathyroid hormone-related protein knockout mouse demonstrates that parathyroid hormone-related protein is essential for mammary gland development. *Development* **125** 1285–1294.
- Yoshiko Y, Son A, Maeda S, Igarashi A, Takano S, Hu J & Maeda N 1999 Evidence for stanniocalcin gene expression in mammalian bone. *Endocrinology* **140** 1869–1874.
- Zhang K, Westberg JA, Paetau A, von Boguslawsky K, Lindsberg P, Erlander M, Guo H, Su J, Olsen HS & Andersson LC 1998 High expression of stanniocalcin in differentiated brain neurons. *American Journal of Pathology* **153** 439–445.
- Zhang K, Lindsberg PJ, Tatlisumak T, Kaste M, Olsen HS & Andersson LC 2000 Stanniocalcin: a molecular guard of neurons during cerebral ischemia. *PNAS* **97** 3637–3642.
- Zheng L, Annab LA, Afshari CA, Lee W-H & Boyer TG 2001 BRCA1 mediates ligand-independent transcriptional repression of the estrogen receptor. *PNAS* **98** 9587–9592.