Iodide transport and breast cancer

Vikki L Poole and Christopher J McCabe

School of Clinical and Experimental Medicine, Institute of Biomedical Research, University of Birmingham, Birmingham B15 2TT, UK

Correspondence should be addressed to C J McCabe
Email
mccabcjz@bham.ac.uk

Abstract

Breast cancer is the second most common cancer worldwide and the leading cause of cancer death in women, with incidence rates that continue to rise. The heterogeneity of the disease makes breast cancer exceptionally difficult to treat, particularly for those patients with triple-negative disease. To address the therapeutic complexity of these tumours, new strategies for diagnosis and treatment are urgently required. The ability of lactating and malignant breast cells to uptake and transport iodide has led to the hypothesis that radioiodide therapy could be a potentially viable treatment for many breast cancer patients. Understanding how iodide is transported, and the factors regulating the expression and function of the proteins responsible for iodide transport, is critical for translating this hypothesis into reality. This review covers the three known iodide transporters – the sodium iodide symporter, pendrin and the sodium-coupled monocarboxylate transporter – and their role in iodide transport in breast cells, along with efforts to manipulate them to increase the potential for radioiodide therapy as a treatment for breast cancer.

Key Words
- breast cancer
- iodide transport
- radioiodide
- sodium iodide symporter (NIS)
- pendrin
- sodium-coupled monocarboxylate transporter (SMCT)

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Introduction

Based on current incidence projections, 3.2 million new cases of breast cancer will be diagnosed each year by 2050 (Hortobagyi *et al.* 2005). Meanwhile, the heterogeneity observed at both the intra- and inter-tumour levels continues to make the disease challenging to treat, particularly for those patients with metastatic triple-negative disease, where few treatment options are available. The inherent ability of breast cells to uptake iodide opens the possibility for a potential alternative treatment for breast cancer via radioiodide therapy, currently used in the management and diagnostic imaging of thyroid disorders.

Although utilisation of radioiodide treatment for breast cancer has been proposed previously, improved functional insight is required before it can be translated from bench to bedside. Crucially, the transport of iodide into breast cells must be maximal while iodide efflux is simultaneously minimised. Full functional understanding of the three major transporters of iodide in breast cells and how they may be manipulated is required for this proposed treatment to become a reality. This review aims to provide an overview of the transporters sodium iodide symporter (NIS), pendrin and sodium-coupled monocarboxylate transporter (SMCT), focusing on factors relating to their expression and function alongside strategies that would maximise their potential in breast cancer.

Sodium iodide symporter

The NIS is a large (643 amino acids) integral plasma membrane glycoprotein (Fig. 1), the primary role of which is transporting iodide (I^-) into cells. The gene, also referred to as solute carrier family 5 member 5 (*SLC5A5*), was first cloned in 1996 (Dai *et al.* 1996), although the ability of the thyroid to accumulate iodide was reported as early as 1896 (Baumann 1896). The protein consists of thirteen transmembrane domains, an extracellular

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N-terminal and a cytosolic C-terminal tail and has been identified to be phosphorylated *in vivo* and contain three N-linked glycosylation sites at positions 225, 485 and 497 (Fig. 1) (Levy *et al.* 1998, Vadysirisack *et al.* 2007).

NIS expression is primarily observed in the thyroid along with salivary glands, gastric mucosa and lactating mammary gland cells, where it is usually located at the basolateral surface of the plasma membrane. In thyroid follicular cells, the cellular concentration of iodide is 20–50 times that of extracellular levels, so use of the inverse Na^+ electrochemical gradient, maintained by the Na^+/K^+ ATPase, allows NIS to couple the transport of one iodide anion and two sodium cations into cells. The NIS-mediated transport of I^- into thyroid follicular cells is the first and rate-limiting step of the biosynthesis of the thyroid hormones triiodothyronine (T_3) and throxyine (T_4) (Spitzweg *et al.* 2001). In the thyroid, the expression of NIS is principally regulated by the thyroid-stimulating hormone (TSH) (Kogai *et al.* 1997). When TSH binds to the TSH receptor (TSHR), adenylate cyclase is activated, leading to increased intracellular cAMP levels (Takasu *et al.* 1978), which further activates the transcription factors, cAMP response element-binding protein (CREB) and Pax8 (Poleev *et al.* 1997). The NIS upstream enhancer (NUE), which is fundamental in the initiation of NIS transcription, contains binding sites for both Pax8 and CREB, which stimulate NIS transcription upon transcription factor binding (Ohno *et al.* 1999).

Only two putative binding partners of NIS have been reported, both of which have been implicated with breast cancer; pituitary tumour transforming gene (PTTG) binding factor (PBF) (Smith *et al.* 2009) and leukaemia-associated RhoA guanine exchange factor (LARG) (Lacoste *et al.* 2012). PBF is a small glycoprotein that shares no significant homology to other human proteins but is widely conserved through a large range of species (Chien & Pei 2000). The upregulation of PBF has been reported in a number of carcinomas, including thyroid, breast and

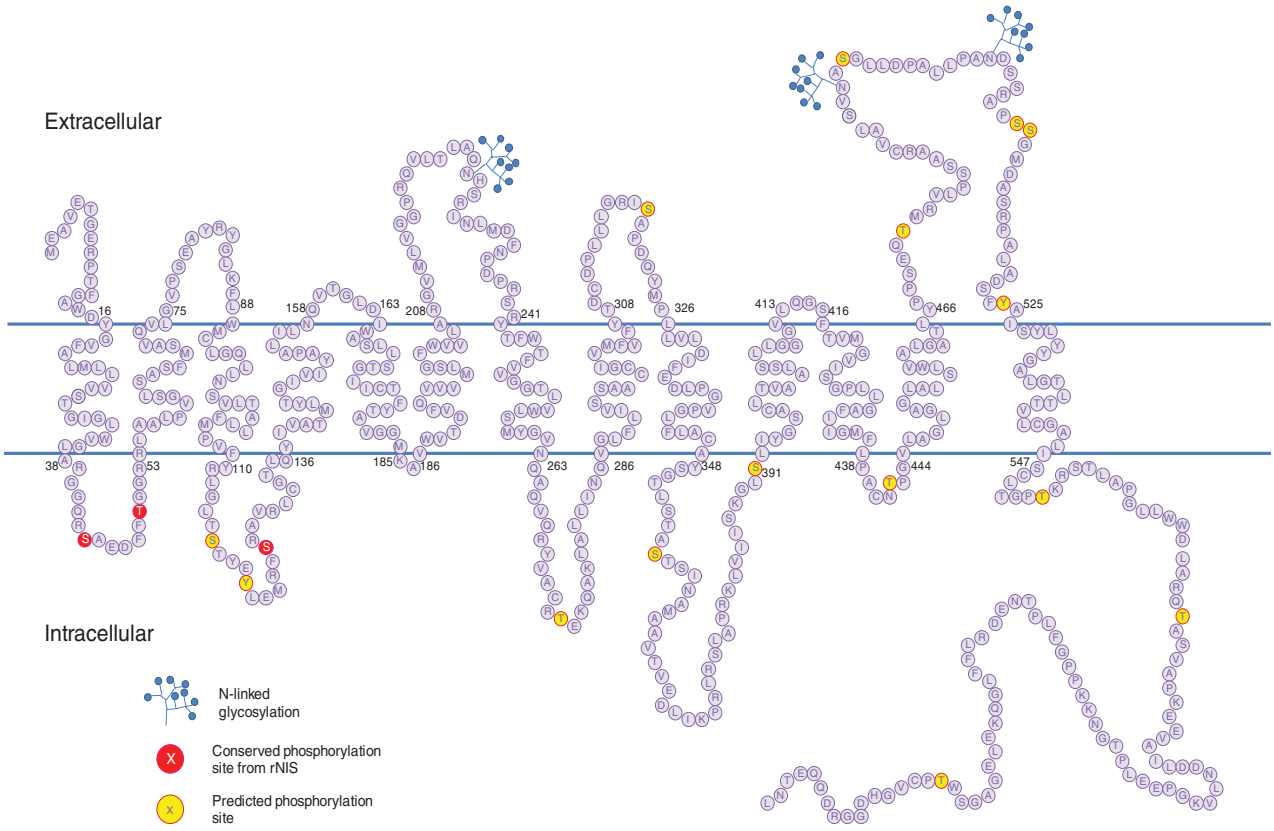

Figure 1
Secondary structure of hNIS. The secondary structure of human NIS with 13 transmembrane domains as predicted by UniProt (http://www.uniprot.org/uniprot/Q92911). NIS is glycosylated at Asn225, Asn485 and Asn497, all found on hydrophilic extracellular loops. Red amino acids are conserved residues that have been observed to be phosphorylated in rat NIS, while amino acids in yellow are those predicted to be phosphorylated by NetPhos2.0.

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colorectal (McCabe *et al.* 2003, Stratford *et al.* 2005, Watkins *et al.* 2010, Read *et al.* 2014). PBF has been reported to repress radioiodide uptake in thyroid cancer cells by binding to NIS, which leads to NIS internalisation and inhibition of function (Smith *et al.* 2009). This interaction is modulated by the phosphorylation of PBF; abrogation of residue Y174 restores plasma membrane NIS and radioiodide uptake (Smith *et al.* 2013). PBF has also been shown to repress NIS transcription at the promoter level, reducing NIS levels and therefore radioiodide uptake (Boelaert *et al.* 2007). LARG is a guanine nucleotide exchange factor for the RhoA GTPase that plays a major role in the reorganisation of the cytoskeleton and cell adhesion. In multiple cancer cell lines, including breast, the interaction between LARG and NIS led to the activation of RhoA, increasing cell invasion and migration. Interaction between NIS and LARG was established to be ion-independent and occur intracellularly, suggesting that the mislocalisation of NIS in many cancers may increase migration of these cells (Lacoste *et al.* 2012), correlating with the observation of NIS at the leading edge of metastatic cells from breast cancer patients (Lacoste *et al.* 2012).

The natural ability of the thyroid to uptake iodide is central to the diagnosis and treatment of hyperthyroidism, Grave's disease and thyroid cancer. This mechanism was exploited as early as 1946 in the treatment of thyroid diseases, including thyroid carcinoma (Seidlin *et al.* 1946). In all, 68–80% of thyroid cancers and their metastases retain functional NIS activity and therefore the ability to accumulate iodide (Castro *et al.* 2001). This uptake has allowed nuclear imaging of the disease using radioiodide 123, 124 and 125 (^{123}I, ^{124}I and ^{125}I) and ablation of malignant tissue using the β-emitting radioiodide-131 (^{131}I) (Spitzweg *et al.* 2001). Ironically, ^{131}I represents a significant public health hazard, implicated in open-air atomic bomb testing in the 1950s, the Chernobyl disaster in 1986 and the Fukushima explosion of 2011. ^{131}I is a major uranium and plutonium fission product, comprising nearly 3% of the total products of fission. Its mechanism is via β decay; iodine-131 is notable for causing mutation and death in cells that it penetrates and other cells up to several millimetres away (the bystander effect). Therapeutic doses therefore differ from atomic fallout in that higher doses kill thyroid cells, whereas lower doses resulting from nuclear contamination initiate mutations, which may then drive tumourigenesis.

Along with iodide accumulation in the thyroid being vital for hormone production, it is also essential that breast-feeding mothers are able to accumulate iodide in their milk. Iodide is an essential constituent of breast milk, with infants who are deficient in iodide having increased risks of impaired neurological development and increased mortality (Cao *et al.* 1994, DeLong *et al.* 1997). This reflects the requirement for thyroid hormones in infants under 1 year of age being higher, by body weight, than at any other time in their lifespan (Delange 1998).

NIS expression in the lactating breast

The ability of humans to accumulate iodide in milk was an observation first identified in 1952 (Honour *et al.* 1952), with NIS being identified as the responsible transporter in 2000 (Cho *et al.* 2000, Tazebay *et al.* 2000). The NIS present in the lactating breast was observed to be functional, with distinct basolateral plasma membrane staining in alveolar cells (Cho *et al.* 2000). In rats, NIS identified in lactating breast was observed to be ~75 kDa in size compared to the 100 kDa form previously observed in rat thyroids. However, the unglycosylated forms observed in both tissues were found at ~50 kDa, suggesting differential glycosylation in thyroidal and lactating breast NIS (Tazebay *et al.* 2000).

The regulation of NIS expression in mammary gland tissue was found to be different from that of the thyroid with NIS, being expressed towards the end of gestation and throughout suckling; however, NIS levels are markedly reduced within 24 h of weaning (Tazebay *et al.* 2000). With the hormones oxytocin and prolactin being heavily associated with lactation, investigations focused on their potential involvement in the regulation of lactating mammary NIS.

As hypothesised, when mice were given doses of oxytocin, they displayed increased accumulation of radioiodide in their milk and elevated NIS expression was observed in mammary tissue (Tazebay *et al.* 2000). This was also confirmed in rats, with an oxytocin antagonist significantly decreasing radioiodide uptake compared to control-treated animals (Cho *et al.* 2000). Prolactin on the other hand, produced varying results. Treatment of mice with prolactin, alone or in combination with oxytocin, did not stimulate NIS expression and was incapable of increasing iodide accumulation in murine milk. However, in rats, treatment with prolactin increased NIS mRNA levels, and bromocriptine, which inhibits prolactin release from the pituitary gland, decreased radioiodide uptake in mammary glands (Cho *et al.* 2000). Both the Tazebay and Cho studies agree that oxytocin and prolactin do not act synergistically, with prolactin actually acting as an antagonist to oxytocin's stimulation of NIS. It was postulated that this was due to prolactin's inhibitory

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effect on steroidogenesis (Dorrington & Gore-Langton 1981), which potentially could decrease estrogen levels below a threshold preventing NIS stimulation by concomitantly administered oxytocin. This hypothesis was supported in ovariectomised mice that, when treated with high levels of exogenous estrogen, prolactin and oxytocin, displayed NIS levels above those without estrogen (Tazebay *et al*. 2000).

NIS expression is thus vital in lactating breasts for the accumulation of iodide, with gene expression being induced by hormones produced by the mother whilst breast-feeding, whereas 'normal' non-lactating breast tissue does not display detectable NIS expression.

NIS in breast cancer

The ability of breast carcinomas to uptake radioiodide was identified as early as 1974, when tumour biopsy tissues were observed to uptake more radioiodide than their normal breast tissue counterparts (Eskin *et al*. 1974). This early study was replicated 2 years later, with a murine study observing high levels of radioiodide uptake within mammary tumours (Thorpe 1976). However, the identification of NIS expression in mammary tumours did not occur until nearly 25 years later, when NIS mRNA was initially detected in six out of seven human breast cancer samples (Kilbane *et al*. 2000), with the identification of NIS protein by immunohistochemistry (IHC) following shortly after (Tazebay *et al*. 2000). Of the total, 87% of breast cancer tumours were reported positive for NIS expression, compared to 0% of the normal non-lactating breast tissues (Tazebay *et al*. 2000). Expression of NIS does not appear to fluctuate between breast cancer tumour types, with tumours such as ductal carcinoma *in situ* and invasive carcinoma having similar NIS levels (Tazebay *et al*. 2000, Wapnir *et al*. 2003). The expression of NIS also appeared to be independent of breast cancer receptor status and has not been correlated to expression of TSHR, ER or PR in breast cancer (Oh *et al*. 2005), although a more recent study did identify a correlation between ER and NIS expression (Chatterjee *et al*. 2013).

Despite NIS expression being identified within breast cancers, only high levels of NIS mRNA expression correlated to measurable NIS functionality, with 17% of NIS-positive tumours being capable of ^{99m}Tc-pertechnetate uptake (Moon *et al*. 2001). The low percentage of tumours capable of radioiodide uptake was confirmed in a later study with only two out of eight of NIS-positive tumours (25%) displaying detectable $^{123}I^-$ uptake (Wapnir *et al*. 2004). The disparity between NIS expression levels and the observed radioiodide uptake led to the hypothesis that NIS may be mislocalised within breast cancer cells. Tazebay *et al*. (2000) initially reported a mixture of cell surface and intracellular staining in cancerous tissue compared to solely basolateral plasma membrane staining observed in lactating breast. Strikingly, a later study identified that only 27% of NIS-positive breast cancers actually have any plasma membrane staining (Beyer *et al*. 2009).

To further investigate NIS expression in breast cancer, 14 genetically engineered mice models of breast cancer were investigated for NIS expression. Mice expressing the oncogene PvMT, *neu*, the human chorionic gonadotrophin (hCG) subunit and Cox2 in mammary glands all strongly immunostained for NIS protein. PvMT, *neu* and Cox2 are capable of activating the phosphatidylinositol-4,5-bisphosphate 3-kinase (PI3K) pathway while hCG and Cox2 can induce cAMP. An investigation into the individual pathways showed that PI3K activation led to increased expression of NIS protein and radioiodide uptake in MCF7 cells, whereas increased cAMP increased NIS promoter activity and mRNA but did not have an effect on radioiodide uptake (Knostman *et al*. 2004). Further investigation into the PI3K pathway suggested that although PI3K activation was capable of increasing NIS expression, it led to an underglycosylated form of NIS and interrupted cell surface trafficking of NIS. Further to this, PI3K levels were positively correlated with NIS expression levels within patient tumours (Knostman *et al*. 2007).

Along with primary breast tumours, the use of radioiodide for locating, monitoring and treating metastases has also been suggested. One study reported that metastatic disease has lower NIS expression, with only 33% of metastases having detectable NIS (Wapnir *et al*. 2004). However, an immunohistochemical study of 28 brain metastases from primary breast cancer tumours reported that 21 (75%) of the tumours were NIS-positive. The NIS-positive tumours were all ER/PR-negative with a mixture of positive and negative HER2 staining. As reported in primary breast tumour, only 24% of the metastasised tumours had plasma membrane staining for NIS, with the rest being primarily intracellular. Although only about a quarter of tumours displayed plasma membrane staining, the observations are promising, as brain metastases, due to the blood brain barrier being impermeable to many chemotherapy reagents, are poor prognostic indicators, with patients having to rely solely on surgery and/or external radiation (Renier *et al*. 2010).

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NIS regulation in breast cancer

As previously discussed, the expression of NIS is under the regulation of TSH in the thyroid. However, this is not the case in breast cancer. It is important to understand what regulates NIS expression in breast cancer to allow potential exploitation of the mechanism for therapeutic purposes. As oxytocin and prolactin are involved in the induction of NIS in lactating mammary glands it was logical to investigate whether they have a role in the expression of NIS in breast cancer. In 3D histocultures of breast cancer tumours, individual treatment with oxytocin and prolactin induced NIS mRNA in a dose-dependent manner. However, a combination of the two hormones was not capable of inducing further NIS expression (Cho *et al.* 2000). Prolactin increases the expression of genes containing gamma-interferon activation sequences (GAS) in their promoter regions, through the activation of the Jak2/Stat5 cascade in breast cells (Burdon *et al.* 1994) (Fig. 2). A GAS sequence has subsequently been identified in the promoter of human NIS (Cho *et al.* 2000). There are conflicting reports as to whether prolactin can increase NIS expression in MCF7s, with Kogai *et al.* (2000) not identifying any stimulation compared to Arturi *et al.* (2005) who observed increased NIS mRNA and iodide-trapping after prolactin treatment. This discrepancy could be due to a variety of technical considerations (Arturi *et al.* 2005). The oxytocin receptor is present in 50–90% of breast cancers (Bussolati *et al.* 1996, Ito *et al.* 1996, Sapino *et al.* 1998) and is a G protein-coupled receptor capable of activating the G_s-cAMP-protein kinase A (PKA) pathway (Olins & Bremel 1984) (Fig. 2). This is the same pathway activated by TSH stimulation in thyrocytes.

Another factor identified to be involved in NIS induction in breast cancers is insulin, along with insulin-like growth factor 1 (IGF1) and IGF2. Insulin and IGF1/2 stimulated NIS expression at both mRNA and protein levels, and increased ^{125}I uptake (Arturi *et al.* 2005). Binding of a ligand to the IGF1 receptor is known to act on insulin receptor substrate 1 (IRS1), which leads to the activation of PI3K (Dupont & Le Roith 2001) (Fig. 2), a pathway associated with NIS expression in breast cells.

Retinoids have been described to be useful in a treatment of a variety of cancers (reviewed in Connolly *et al.* (2013)) with systemic retinoids being FDA-approved for treatment of cutaneous T cell lymphoma (Duvic *et al.* 2001) and acute promyelocytic leukaemia (APL) (Tallman *et al.* 1997). Kogai *et al.* hypothesised that retinoic acid (RA) may be capable of inducing NIS expression in MCF7 cells, a cell line that expresses both RA receptors (RARs) and retinoid

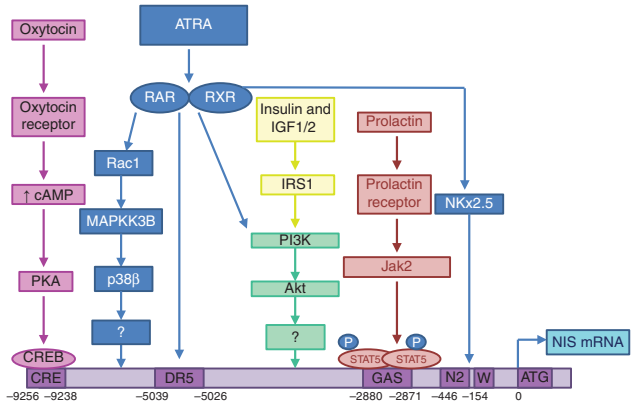

Figure 2
NIS gene regulation in breast cancer. NIS gene expression is upregulated in breast cancer by factors including oxytocin, ATRA, insulin, insulin-like growth factor 1/2 (IGF1/2) and prolactin. Oxytocin binds to the oxytocin receptor, a Gs-coupled receptor increasing intracellular levels of cAMP resulting in the activation of protein kinase A (PKA) pathway. PKA phosphorylates the transcription factors cAMP response element binding protein/cAMP responsive element modulator (CREB/CREM), which bind to the CRE-like sequences in the NUE, increasing levels of NIS expression. Upon insulin and IGF1/2 binding to their receptors, insulin receptor substrate 1 (IRS1) binds to the intracellular regions of the receptor and becomes phosphorylated. This leads to the phosphatidylinositol-4,5-bisphosphate 3-kinase (PI3K) pathway becoming activated and increased levels of pAkt. Elevated levels of pAkt have been associated with an upregulation of NIS in breast cancer, although the mechanism has not yet been further elucidated. Prolactin binds to the prolactin receptor causing dimerisation of two receptors and leading to Janus Kinase (JAK2) recruitment on the cytoplasmic regions of the receptor. Activation of JAK2 at the prolactin receptor leads to STAT5 phosphorylation and dimerisation. STAT5 dimers associate with gamma-interfon activation sequences (GAS) in the promoter region of NIS stimulating transcription of the gene. All-trans retinoic acid (ATRA) is capable of increasing NIS expression through a variety of mechanisms. ATRA binds to retinoic acid receptor (RAR) causing dimerisation with retinoid X receptor (RXR); this heterodimer can directly bind to retinoic acid response elements (RAREs) on target genes. Although NIS does not have any full RARE sequences upstream of the ATG start codon, it does contains a DR5 sequence within its promoter which is responsive to ATRA, and binding of the RAR/RXR heterodimer to this region can stimulate transcription of NIS mRNA. NKx2.5 is important in ATRA's induction of NIS. ATRA induces NKx2.5 expression which subsequently binds to N2 and W regions (defined in Dentice *et al.* (2004)) in the promoter region of NIS, resulting in increased NIS expression. Activated RAR/RXR heterodimers have been observed to directly interact with PI3K and initiate induction of the pathway in the same manner observed with insulin induction of NIS. RAR/RXR dimers are hypothesised to interact directly with Rac1, a small GTPase, leading to MAPK kinase 3B (MAPKK3B) phosphorylating p38, which through an unknown transcription factor stimulates NIS expression.

X receptors (RXRs). In MCF7s, all trans-RA (ATRA) increased ^{125}I uptake in a dose dependant manner that could be inhibited by potassium perchlorate suggesting that uptake was mediated specifically via NIS. Treatment with ATRA increased NIS expression at both the mRNA and protein level, with subsequent ^{131}I treatment reducing colony formation in MCF7 cells. MCF12A cells (derived from normal breast tissue) and ER-negative MDA-MB231 cells

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did not show such a marked response to ATRA treatment, with NIS protein expression being only slightly stimulated in MDA-MB231s and treatment having no effect on iodide uptake in either cell line (Kogai *et al.* 2000). This disparity between cell lines is most likely due to MDA-MB231 cells being RA 'resistant' due to decreased expression of RARα and RARβ compared to MCF7 cells (Liu *et al.* 1996). Subsequently, mice bearing MCF7 xenograft tumours were utilised to establish whether systemic retinoids were capable of the same NIS induction witnessed *in vitro*. Tumour-bearing mice treated with a time release pellet containing ATRA for 5 days prior to ^{125}I treatment accumulated 15 times more radioiodide than control treated mice and displayed increased NIS expression. ATRA was also capable of increasing iodide uptake approximately twofold in a transgenic breast cancer mouse model (murine mammary tumour virus-polyoma virus middle T antigen (MMTV-PyVT)-transgenic mice) (Kogai *et al.* 2004). Further studies established that RARβ/γ stimulation by retinoids led to the most pronounced increase in radioiodide uptake, whereas stimulation of RARα, RARγ and RXR only lead to a modest increase in uptake. Dexamethasone (Dex) was discovered to synergistically increase NIS expression and radioiodide uptake alongside ATRA in MCF7 cells. Dex treatment alone increased NIS mRNA levels slightly but when used in conjunction with ATRA, NIS mRNA increased over 70-fold, with Dex increasing the stability of NIS mRNA (Kogai *et al.* 2005). These findings were later confirmed with MCF7 cells treated with ATRA and Dex in combination having significantly decreased cell survival after ^{131}I treatment compared to ATRA alone treated cells (Unterholzner *et al.* 2006).

ATRA and other retinoids are capable of binding to RARs that heterodimerise with RXR. The heterodimer then binds to RA response elements (RAREs) on target genes and stimulates transcription. Investigation into the NIS promoter in MCF7 cells revealed there were no putative full RAREs. However, several DR2 element sequences with typical half-sites were identified between the first and thirteenth introns of NIS (Kogai *et al.* 2008). These DR2 sites were unresponsive to ATRA, although a DR5 element located in the promoter region of NIS was responsive to ATRA in MCF7 cells (Kogai *et al.* 2008) (Fig. 2). Binding of RARα to the intronic DR2 elements was later reported within 30 min of treatment of MCF7 cells with ATRA (Alotaibi *et al.* 2010), suggesting these DR2 elements have a potential role in the induction of NIS (Kogai & Brent 2012). However, the introns of murine NIS do not contain DR2 elements yet are still capable of ATRA induced NIS expression in transgenic mice models (Kogai *et al.* 2004), suggesting other non-traditional mechanisms of induction may be apparent.

One potential mechanism is through homeobox protein Nkx2.5, which is induced following ATRA treatment. Use of Nkx2.5 mutants suggested that Nkx2.5 is critical for ATRA's induction of NIS and radioiodide uptake (Dentice *et al.* 2004) (Fig. 2). A range of inhibitors was utilised to try to identify which pathways are critical for ATRA mediated induction of NIS expression and identified that the activities of IGF receptor, PI3K, JAK, and p38 MAPK were important (Kogai *et al.* 2008). It is established that natural retinoids are capable of stimulating the IGF receptors (Kang *et al.* 1997) and that activated RAR/RXR heterodimers can interact with IRS1 (del Rincon *et al.* 2003). It was hypothesised that RAR, through its interaction with IRS1, can activate the PI3K pathway leading to NIS expression (Kogai *et al.* 2008). Activated RAR/RXR heterodimers have also been shown to directly activate PI3K with p85, a sub-unit of PI3K, observed to bind the heterodimers (Fig. 2). Silencing/inhibition of p85 led to a reduced NIS expression after ATRA treatment (Ohashi *et al.* 2009) suggesting that the PI3K pathway plays an important role in ATRA mediated NIS induction.

The p38 MAPK pathway was also associated with ATRA's induction of NIS (Kogai *et al.* 2008) and ATRA stimulated RAR/RXR heterodimers have been observed to activate the p38-Rac1 pathway (Alsayed *et al.* 2001). Inhibition of p38 was noted to reduce NIS at both the basal level and with ATRA treatment (Kogai *et al.* 2008) and the use of inhibitors and siRNA demonstrated that Rac1, MAPK kinase 3B (MAPKK3B) and p38β were all required for the full induction of NIS by ATRA in MCF7 cells (Kogai *et al.* 2012). It is hypothesised that ATRA stimulates dimerisation of RAR/RXR, which interacts with the small GTPase Rac1, leading to MAPKK3B phosphorylating p38. The phosphorylation of p38 leads to the induction of NIS transcription through unknown factors in breast cells (Kogai *et al.* 2012) (Fig. 2).

Although the use of prolactin, oxytocin, insulin and retinoids has been identified to induce NIS expression within breast cancer tissues, the mechanisms are not fully elucidated and the complications of these systemic treatments are not fully overcome. Thus, efforts have been made to try to enhance NIS levels and radioiodide uptake by exogenously expressing NIS.

NIS gene therapy in breast cancer

Along with the induction of endogenous NIS, another method of increasing NIS expression in breast cancer cells

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is via exogenous expression. Retroviral expression of NIS in murine and human tumour cells induced uptake of radioiodide in xenograft models, with *in vitro* uptake of ^{131}I being sufficient to kill cells (Mandell *et al*. 1999). To identify whether NIS could be exogenously targeted to pre-existing tumours, xenograft SiHa and MCF7 tumours were established in murine models before NIS adenoviral plaque-forming units (PFU) were injected directly into the tumour. The injected tumours were observed to be capable of 25-fold higher radioiodide uptake than their control counterparts. However, after treatment with ^{131}I, there was no difference in tumour size between control and NIS-transfected tumours, suggesting that the tumour retention time of radioiodide was not sufficient to inhibit cell growth or ablate tumour cells (Boland *et al*. 2000).

To further increase NIS expression in tissues, NIS can be exogenously expressed within cells under promoters specific to certain cell types. LNCaP prostate cancer cells were stably transfected with NIS under the control of the prostate specific antigen (PSA) promoter. Xenografts of these cells were transplanted into mice and treated with a single i.p. injection containing 3 mCi ^{131}I. Mice with tumours expressing NIS had a significantly reduced tumour size compared to control tumours (Spitzweg *et al*. 2000). Subsequently, breast tumours were investigated using NIS under control of the MUC1 promoter. MUC1 is a glycoprotein usually observed in haematopoietic cells, although expression is observed in ~90% of breast cancers and has been correlated with poor survival and increased metastasis (Gendler 2001). MUC1-positive T47D cells displayed a 58-fold increase in radioiodide uptake after adenoviral transduction with NIS compared to control transfected cells, whereas MUC1-negative MDA-MB231 cells showed no increase in radioiodide uptake after transduction. *In vivo* studies with T47D xenograft tumours established in mice were injected at multiple sites with adenoviral NIS and treated with an i.p. injection of 3 mCi of ^{131}I. NIS expressing tumours were reduced in size by ~83% whilst control tumours continued to increase in size (Dwyer *et al*. 2005). Use of adenoviral expression of NIS under the control of an estrogen receptor responsive promoter (4ERE) was also successful in increasing radioiodide uptake and inhibited tumour growth after ^{131}I treatment in ER-positive breast cancer cells.

It has previously been reported that one of the limitations of gene therapy in a preclinical and clinical setting is the difficulty in detecting gene expression following administration. For clinical settings in particular, a non-invasive and reproducible detection method is required to establish location, magnitude and kinetics of gene expression (Tjuvajev *et al*. 1995). In this context, NIS is advantageous as it can be easily and non-invasively detected using gamma scintigraphy with ^{123}I or $^{99}TcO_4^-$ or positron emission tomography (PET) with ^{124}I and ^{76}Br to image where it has successfully been expressed. Studies have also been conducted with the use of NIS as a reporter gene for the transfection of tumour cells with p53 and manganese superoxide dismutase (MnSOD) (Niu *et al*. 2006).

Use of RA was also capable of increasing adenoviral NIS expression when NIS was under control of the CMV promoter in MCF7 cells (Lim *et al*. 2007). The CMV promoter contains RAREs (Angulo *et al*. 1996), which can be bound by stimulated nuclear RARs endogenously present in MCF7 cells (Titcomb *et al*. 1994). The use of the human telomerase reverse transcriptase promoter also proved effective for increasing iodide uptake and ablation in breast cancer cells (Riesco-Eizaguirre *et al*. 2011).

Along with the use of retro- and adenoviruses, oncolytic viruses have been used as a form of delivery system for exogenous targeting of proteins. GLV-h153, an oncolytic vaccinia virus carrying hNIS, was injected into mice xenograft tumours, leading to increased visualisation of the tumours with ^{124}I. Subsequent treatment with ^{131}I also suppressed tumour growth compared to control (Gholami *et al*. 2014).

Although NIS gene therapy appears effective, an efficient and optimal method of delivery of the gene to the tumour site has not been established. Use of superparamagnetic iron oxide (SPIO)-labelled AC133+ progenitor cells (APCs) transduced with adenoviral hNIS in mice with MDA-MB231 xenograft tumours increased ^{99m}Tm activity at the site of the tumour. The labelled APCs were administered to the mice intravenously and images taken using MRI and single photon emission computer tomography (SPECT) showed accumulation of the labelled cells at the site of the tumour along with NIS expression (Rad *et al*. 2009). The use of mesenchymal stem cells (MSCs), which have been shown to migrate specifically to tumours *in vivo*, has been suggested for gene therapy (Spaeth *et al*. 2008, Dwyer *et al*. 2010). MSCs adenovirally infected with NIS were intravenously injected into mice bearing MDA-MB231 xenograft tumours. Three days after i.v. injection of the MSCs, uptake of ^{99m}Tc was detected at the site of the tumour using SPECT and remained detectable 14 days after injection. NIS expression was detected in tumour tissue, and at day 14 mice treated with ^{131}I had a significant reduction in breast xenograft tumour size compared to control mice (Dwyer *et al*. 2011).

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Pendrin

Pendrin, coded by the solute carrier family 26 member 4 gene, (*SLC26A4*) *PDS*, is a sodium-independent chloride/iodide transporter (Everett *et al*. 1997) and is found to be abundantly expressed in the thyroid and the inner ear. Like NIS, pendrin is a large (780 amino acids) transmembrane protein that spans the plasma membrane multiple times (Everett *et al*. 1997). Initial studies found that in the thyroid, pendrin was capable of mediating the transport of iodide and chloride (Scott *et al*. 1999), with pendrin being located on the apical membrane of thyrocytes (Bidart *et al*. 2000) mediating iodide efflux (Yoshida *et al*. 2002). Supporting these *in vitro* data, patients suffering Pendred syndrome, a disease where the gene-encoding pendrin (*PDS*) contains an inactivating mutation, display altered iodine organification (Sheffield *et al*. 1996). However, more recently there have been questions as to pendrin's role in iodide transport as patients with biallelic PDS mutations, with sufficient iodide intake, displayed no altered thyroidal phenotype (Sato *et al*. 2001) and pendrin knockout mice did not develop goitres and had normal thyroid function tests (Everett *et al*. 2001).

In lactating mammary glands, an iodide/anion exchanger that was sensitive to 4,4′-diisothiocyano-2,2′-stilbenedisulfonic acid (DIDS) was discovered (Shennan 2001) and was later identified as pendrin (Rillema & Hill 2003*a*). Akin to NIS, there is little to no expression of pendrin in normal mammary glands, but it is induced in lactating breast. Pendrin expression was shown to be stimulated by prolactin, increasing levels of iodide uptake, which were returned to normal levels in the presence of several pendrin inhibitors, including DIDS. Although the data were not shown, Rillema and Hill claimed lactating tissue provided the highest expression of pendrin in immunohistochemical studies, with the staining being specific to alveolar epithelial cells with none detected in stromal cells. However, it was then stated that there was no specific localisation of pendrin to the apical or basolateral surface of the alveolar epithelial cells, and so no comment could be made on the localisation of functional pendrin that was observed (Rillema & Hill 2003*a*).

Following identification of pendrin in the lactating breast, subsequent studies were performed to identify potential pendrin expression in breast cancer. Pendrin expression was confirmed in MCF7 cells by western blotting. Radioiodide uptake in MCF7 cells was shown to be decreased in cells treated with DIDS but not sodium perchlorate or ouabain. This decrease was shown to be sodium-independent, suggesting that pendrin was responsible for iodide uptake, not NIS as previously observed. From these findings the authors went on to suggest that differences between batches of MCF7 cells or culturing conditions could potentially result in variable expression of iodide transporters (Rillema & Hill 2003*b*). A later study also found that in the mammary glands of rats treated with the carcinogen, N-methyl-N-nitrosourea (MNU), pendrin expression was increased when cells were treated with iodine (I_2) (Garcia-Solis *et al*. 2005).

Although pendrin expression has been reported in MCF7s and rat breast tumours, further work is required to elucidate whether there is widespread pendrin expression within breast cancer. Currently there is little evidence to suggest that pendrin is a major player in breast cancer, let alone iodide transport within the disease.

Sodium-coupled monocarboxylate transporter

The SMCT, also referred to as apical iodide transporter (AIT), is encoded by the SLC5A8 gene and is usually located on the apical membrane of thyrocytes, transporting iodide from thyrocytes to colloid lumen (Rodriguez *et al*. 2002). SMCT is a ~69 kDa protein that shares 46% homology with NIS and is downregulated in many carcinomas, including thyroid cancer (Lacroix *et al*. 2004, Porra *et al*. 2005), and has thus been suggested to be a tumour suppressor gene (Porra *et al*. 2005). SMCT is abundantly expressed in the colon (Li *et al*. 2003) and functions as a Na^+/short-chain fatty acid co-transporter (Miyauchi *et al*. 2004). High expression levels of SMCT have been reported during mammary gland involution and in normal breast epithelial cells (Gupta *et al*. 2006). However, SMCT is significantly downregulated in breast cancer cell lines and in primary breast tumours, with MCF7 cells reported to have no expression (Thangaraju *et al*. 2006).

Due to its lack of expression in breast cancer, this transporter plays no reported role in the iodide transport of breast cancer cells. Even though SMCT is hailed as a tumour suppressor, given its role in iodide efflux within the thyroid, expression of SMCT within breast cancer could be counterintuitive for the proposed radioiodide therapy. Although studies have reported a lack of expression of SMCT in breast cancer, it may be important to further investigate the role of SMCT in iodide transport within normal breast tissue. Observations from these studies could be important in identifying changes in iodide transport in breast cells progressing to cancer, where SMCT expression is switched off.

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Conclusion

Understanding the roles of the three known iodide transporters within breast cancer is of the utmost important for translating the proposed use of radioiodide therapy from the bench to the bedside. From the evidence discussed, the major iodide transporter in breast cancer is NIS, with pendrin potentially having a more minor role. Although NIS is expressed in the majority of breast cancers and metastases, the inhibitory issue for radioiodide therapy is the intracellular localisation of NIS, rendering it non-functional. There is an obvious need for new studies to discern mechanisms of increasing functional NIS localisation. The two prospective ways of doing this are by increasing total NIS levels or by manipulating cells to alter the trafficking of NIS.

The use of retinoids to increase endogenous NIS expression has proved promising *in vitro*, but concerns over the systemic effects associated with retinoid use need to be carefully considered. While exogenous expression of NIS in cells has proved successful *in vivo*, delivery methods for gene targeting are not fully optimised and further investigation and trials are required before this is a viable solution. Although increasing total NIS levels is often championed, there are potential problems that could be associated with this method of increasing iodide transport. An increase of total NIS may lead to a serendipitous increase of plasma membrane NIS, but it also may be associated with a rise in intracellular NIS levels, which has previously been correlated to increased migration and invasiveness through its interaction with LARG (Lacoste *et al.* 2012). Alternative studies into manipulating the subcellular location of NIS, using established and potentially novel binding partners should also be heavily considered. PBF has been observed to decrease plasma membrane expression of NIS in thyroid cancer (Smith *et al.* 2009), so a similar mechanism may exist within breast cancer, with the potential for inhibition of phosphorylation of PBF being capable of restoring plasma membrane expression.

Overall, the potential for the use of radioiodide treatment in breast cancer is one that should be continued to be investigated. The regulation and subcellular location of NIS appears to be unique in breast cells, so further investigations within normal, lactating and malignant tissues are required. Although NIS appears to be the protein responsible for the majority of iodide transport within breast cancer, SMCT and pendrin should not be overlooked and their roles within the disease should continue to be elucidated.

Declaration of interest

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

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