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The Neuregulin Family of Genes and their Multiple Splice Variants in Breast Cancer

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Abstract The neuregulin family consists of four genes, *NRG1–4* which can each encode products containing a domain related to the epidermal growth factor family of ligands. Each gene is subject to complex control of transcription and to splicing of their mRNA product to give many variant proteins. These do not contain secretory sequences but some, through their transmembrane sequence, are routed via the Golgi where they are glycosylated, to the cell surface. Here they may be released by regulated proteolysis to act as soluble proteins which can interact and activate members of the EGF receptor family of receptor tyrosine kinases. Other splice variants do not encode transmembrane sequences and these are found either in the cytoplasm or, if they encode a nuclear localisation sequence, in distinct compartments in the nucleoplasm. It has been shown that the variants containing a full EGF domain can act as receptor agonists but the function of the cytoplasmic and nuclear products is unknown as yet. All four neuregulin genes are expressed and play an important role in mammary gland development. They are also expressed at elevated levels in some cases of ductal carcinoma in situ of the breast and breast cancer. They seem to be active in this setting and their presence may affect the efficacy of treatment with endocrine agents or with signal transduction inhibitors directed at the EGF receptor family members. Much remains to be learned however of their normal function and their influence on breast cancer development, progression and response to therapy.

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Abbreviations

EGF	epidermal growth factor
TGF-α	transforming growth factor alpha
HER	human epidermal growth factor receptor

Introduction

The ErbB receptor tyrosine kinase family and their cognate ligands can promote a wide range of biological events including the induction and progression of several epithelial cancers. This receptor family consists of four members known as ErbB1 (also designated as epidermal growth factor (EGF) receptor or HER1), ErbB2 (HER2), ErbB3 (HER3) and ErbB4 (HER4). The monomeric receptors are activated by extracellular EGF-like ligands which facilitate dimerisation. This allows for either receptor homodimerisation or heterodimerisation and subsequent activation of the intracellular tyrosine kinase domain. Inter-receptor transphosphorylation events initiate intracellular signalling cascades that can direct both normal processes in mammalian development, including cell migration, apoptosis and differentiation, and are involved in the pathology of several diseases including cancer [1] and neurological disorders such as schizophrenia [2].

There are 11 ligands so far described which can be classified into four groups, those that interact with EGF receptor only (EGF, Transforming Growth Factor Alpha (TGFα), and amphiregulin), those that bind to EGF receptor and the HER4 receptor (heparin binding-EGF, betacellulin and epiregulin), those which bind directly to either HER3 and HER4, (neuregulin 1 and neuregulin 2)

and those which only bind to HER4 (neuregulin 3 and neuregulin 4) [3]. Epigen, the most recently discovered member of the family appears to be a low affinity ligand with broad specificity for ErbB receptor binding [4]. The EGF domain is the second most commonly identified in the human genome being present in more than 3,000 different genes (http://www.sanger.ac.uk/Software/Pfam/) and thus it is possible that further ligands exist. It has been suggested that tomoregulin 1 and 2 [5, 6] and neuroglycan C [7] are also potential ligands but this remains to be confirmed definitively. Stein and Staros [8] have defined genes encoding potential EGF-like ligands as containing a splice site between the fourth and fifth cysteines and a nearby potential transmembrane sequence and from this identify several additional gene families, including interphotoreceptor proteoglycan-2, the alpha and beta subunits of meprin A and mucins 3,4,12 and 17 (as well as the tomoregulins 1 and 2 and neuroglycan C). As yet there is little experimental data published on receptor binding or biological assays with these putative ligands, which will be required to induct them into, or exclude them from the family.

In addition, it should be remembered that ligand specificity is defined by the affinity of binding of each ligand for individual receptors but that this does not necessarily relate to their biological activity. For example epigen binds with 100 fold lower affinity than EGF to the EGF receptor yet it is a more potent mitogen, probably because it evades degradation and is inefficient at down-regulating its receptor [4].

This short review will explore the gene and protein structure of the four neuregulins and correlate this with their possible functions and sub-cellular localisation. Further discussion will include how the members of this diverse family of EGF-like ligands are involved in the development of the normal breast and breast cancer and the implications which arise for their influence on the treatment of breast cancer.

Neuregulins 1–4

The neuregulins (NRGs) are a large, diverse family of EGF-like ligands encoded by four genes *NRG1–4* which are subject to extensive and complex alternative mRNA splicing. Evolutionary relationships defined by sequence comparisons of their EGF domains segregates the neuregulin genes into a sub-family in which NRG4 diverged first, followed by NRG3 and then the pair of neuregulins 1 and 2 which are the most highly conserved [8].

The first neuregulin to be discovered, NRG1, was identified separately in quite different systems emphasising its diverse roles (see [9] for a comprehensive review). NRG2 was identified by PCR using primers from NRG1 ([10], called NTAK) or by bioinformatic searches ([11], called Don-1). NRG3 [12] and NRG4 [13] were identified by bioinformatic searching of the sequence databases. The *NRG1* gene [14] and individual exons have been knocked out in mice, as has NRG2 [15]. Loss of expression of the complete array of *NRG1* gene products was embryonic lethal while NRG2 knockouts survived, but with early growth retardation and reduced reproductive capacity. The effects of knocking out NRG3 or NRG4 have not yet been reported.

The NRG1 isoforms are involved in the development and function of a diverse range of tissues including the nervous system [16], heart, vascular endothelial cells [17] and in mammary gland formation [18]. In particular NRG1 is involved in a large number of neuronal functions but it is only recently that we are beginning to elucidate and discriminate between the functions of the other neuregulins, NRG2, NRG3 and NRG4 and their splice variants. NRG2 is widely expressed in the developing nervous system [19], and in other embryonic tissues outside of the central nervous system including the lung, heart and bladder. It is also expressed in discreet areas of the adult brain, the cerebellum, the dendate gyrus and the olfactory bulb [11, 19, 20–22]. NRG2 is synthesised by Schwann cells and motor neurones and is concentrated at synaptic sites [23]. NRG3 is expressed in the human embryonic and adult central nervous system [12, 24] and NRG4 is expressed at the mRNA level in pancreas and muscle [13] and at the protein level in human breast cancer [25] and prostate cancer [26]. Using antibodies to each of the EGF domains of NRG 1–4 we have observed expression in many normal human organs and cell types beyond those mentioned above (Gullick, unpublished). This growing list emphasises the range of tissue and cell types whose behaviour is likely to be influenced by neuregulin signalling.

Splicing of NRG Genes

NRG1

The extraordinary diversity of the neuregulin signalling pathway is achieved by extensive alternative splicing of the *NRG1* gene which, together with the use of at least six transcription initiation sites (and possibly nine promoters) [8, 27] gives rise to the production of more than 15 protein variants [9]. These variants are tissue-specifically expressed, presumably due to alternative promoter usage and differ significantly in their structure, due to alternative splicing and post-translational modification. The type I sub-group of NRG 1 isoforms (originally known as the heregulins) have a unique N-terminal sequence which is

followed by an immunoglobulin-related sequence and then an EGF-like domain. The type II variants (termed glial growth factors) contain an N-terminal kringle-like sequence, the immunoglobulin domain and then the EGF-like domain. The type III forms contain an N-terminal hydrophobic domain within a cysteine-rich region, omit the immunoglobulin domain and then continue into the EGF element and various downstream alternative exons. The type II and type III unique N-terminal sequences determines whether these transmembrane isoforms are proteolytically cleaved from the extracellular surface of the membrane.

In all these variants the last third of this EGF sequence can be either spliced into the α or β exon which confers to the β isoforms a 10 to 100 fold greater affinity for their receptors [28]. Downstream from the EGF domain, the neuregulins may contain a linker sequence close to the extracellular surface of the cell membrane that includes a site for proteolytic attack by a member of the ADAM (*a* *d*isintegrin *a*nd *m*etalloproteinase) family of membrane associated enzymes. In particular it has been shown that ADAM 17 (also known as tumour necrosis factor α-converting enzyme or TACE) is implicated in the cleavage of transmembrane neuregulin precursors [29]. High levels of expression of ADAM-17 in breast cancers is associated with shorter overall survival [30] and may be a target for new therapies [31, 32].

Some NRG1 isoforms lack a transmembrane region and appear to be soluble [33] including NRG1β3 which translocates to the cell nucleus (see below) while others continue with a hydrophobic, transmembrane, sequence, and a variable C-terminal tail.

NRG2

The human *NRG2* gene consists of 15 exons which can encode an N-terminal sequence followed by an immunoglobulin-related domain then the EGF-like sequence, a transmembrane sequence and cytoplasmic sequences [22, 34] (Fig. 1a). Exons 1, 2 and 3 contain consensus ATG translation initiation codons and exons 9, 10 and 15 stop codons. Alternative splicing allows for the production of at least nine transcripts [10, 11, 20–22] including alternate α and β C-terminal regions of the EGF domain. These, when expressed as recombinant proteins had different effects on phosphorylation of the ErbB3 and ErbB4 receptors [35, 36]. Yamada et al. [22] also report mRNAs with both α and β regions present or neither (the latter has been called the γ form, which is also reported as a product of the *NRG1* gene [9] and the *NRG4* gene [26]). Other forms terminate prior to the transmembrane domain but their subcellular location has not been reported (Fig. 1a). Yet further splicing leads to the presence or absence of a nine amino acid sequence located just outside the cell membrane reminiscent of the alternative sites of proteolytic cleavage characterised in NRG1 [11].

NRG3

NRG3 was discovered independently by two groups both using bioinformatic searches [12, 37]. These first two reports identified different C-terminal sequences suggesting alternative splicing, as in the *NRG1* and *NRG2* genes. Subsequent analysis showed that the mouse gene apparently contained ten exons [38] but Carteron et al. [24] later identified two additional 5′ exons in the human gene in mRNA from foetal brain (termed hFBNRG3) (Fig. 1b). In the hNRG3 isoform exon 1 is spliced into exon 4 and in hFBNRG3 exon 2 is spliced into exon 3. The hNRG3 isoform has a transcription initiation site in exon 1 and hFBNRG3 in exon 2 producing two unrelated N-terminal sequences of 275 or 54 amino acids. The gene can therefore encode alternative N-terminal sequences followed by an EGF-like domain, a transmembrane domain and a large C-terminal sequence containing a coiled-coil region and a di-leucine motif which the authors suggest may have a role in DNA binding, protein–protein interactions or protein sorting or trafficking [24]. Neither contains an extracellular immunoglobulin-related or kringle domain.

Howard et al. [38], using primers from exon 1, identified several different splice variants including the presence or absence of exon 4, which encodes a short sequence just upstream from the transmembrane region, now giving the familiar indication that this may affect sensitivity of the product to proteolytic cleavage. Other major differences include the inclusion or not of exon 9 and a number of more subtle variations including different numbers of bases at the start of exons 4 and 8. It is possible yet further exons may be found by studying EST databases or by more sequencing as any products which contained shorter sequences would not have been detected by the PCR approach used. In our own work using bioinformatics and PCR (Smart and Gullick, unpublished) we have preliminary evidence of several other splice variants including some which contain stop codons upstream of the EGF domain.

NRG4

Five alternatively spliced isoforms of NRG4 (NRG4 A1, NRG4 A2, NRG4 B1, NRG4 B2 and NRG4 B3) have been described, all of which have the same translation initiation site in exon 1, and also contain exon 2 which encodes the first two thirds of the EGF element [26] (Fig. 1c). The unique C-termini of each NRG4 isoform are produced by alternative splicing between exons 3 to 9. NRG4 A1 and

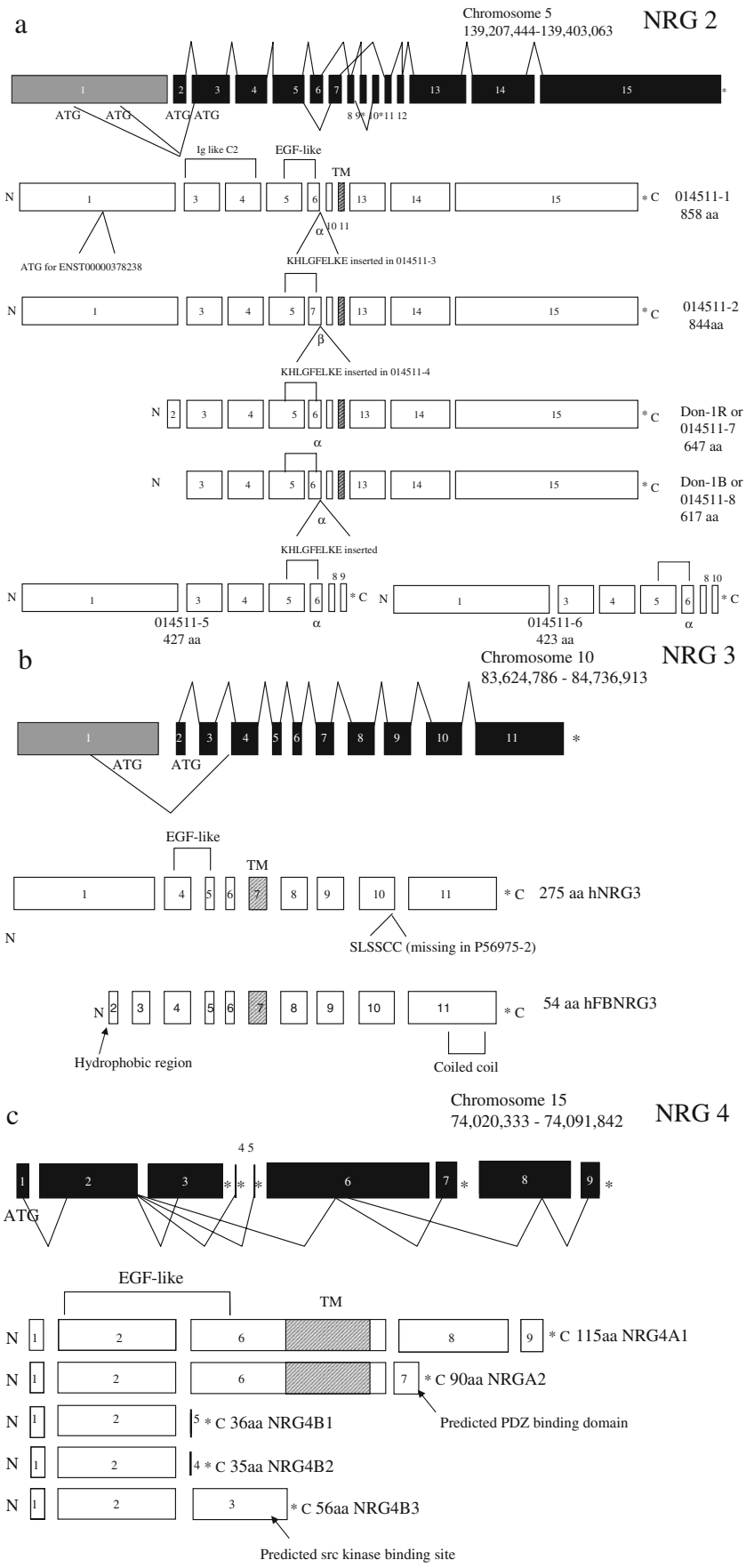

Figure 1 Transcript alignment and splice variant comparison for human NRG2, 3 and 4. **a** Human NRG2 (GenBank accession number XM_001129975) contains 15 exons encoding at least nine separate transcripts. Exon 1 allows translation from two separate initiation codons as indicated. The second ATG encodes for ENST00000378238 (UniProt ID number). In addition, the initiation codon in exon 2 encodes for variant 014511-8 (UniProt) or Don-1R and the initiation codon in exon 3 for variant 014511-7 or Don 1B [11]. An Ig-like C2 type motif is encoded by exons 3 and 4. Alternative splicing which splices into exon 6 produces an α-EGF domain while splicing into exon 7 produces a β-EGF domain. A potential transmembrane (*TM*) region is encoded by exon 11 which is present in all transcripts except 014511-5 and 014511-6 which are designated as the α*1 and α*2 isoforms by Ring et al. [34]. Extensive alternative splicing at the 3′ end of exons 6 and 7 produce alternative splice variants as indicated. Stop codons are indicated by *asterisks*. **b** Human NRG3 (GenBank accession number AK_098823) either encodes hNRG3 in which exons 2 and 3 are spliced out or hFBNRG3 (human fetal brain NRG3) in which exon 1 is spliced out. hFBNRG3 contains an additional hydrophobic region in exon 2. Exons 4 and 5 encode an EGF-like domain and exon 7 a predicted transmembrane (*TM*) domain. **c** Human NRG4 (GenBank Accession number BC_017568) encodes five isoforms. NRG4 A1 and NRG4 A2 both contain a complete EGF-like domain and a putative transmembrane region encoded by exons 2 and 6. There is a predicted PDZ domain at the 3′end of exon 7 in NRG4 A2. The NRG4 B-forms lack exon six and thus encode partial EGF-like domains. NRG4 B3 contains a potential src kinase phosphorylation site in exon 3.

NRG4 A2 both possess predicted transmembrane sequences [13, 26] but the three NRG4 B isoforms do not [26]. NRG4 A1 and NRG4 A2 contain exons 2 and 6 which encodes the intact EGF element and the transmembrane domain. In addition, NRG4 A1 contains exons 8 and 9 which encode an intracellular sequence. NRG4 A2 does not use exons 8 and 9 but splices to exon 7 which encodes six unique C-terminal amino acids which conform to a class I PDZ motif. NRG4 BI is spliced from exon 2 into exon 5 and this encodes a single lysine residue, NRG4 B2 is spliced from exon 2 into exon 4 which encodes a serine, and NR4 B3 is spliced from exon 2 into exon 3 which encodes a 21 amino acid C-terminal sequence which contains a predicted non receptor tyrosine phosphorylation site [26]. NRG A1 and 2 are transported to the cell membrane where they may be released by an ADAM/TACE type enzyme as active soluble ligands [39]. The B forms are retained within the cytoplasm.

Glycosylation

All neuregulin genes express products that are capable of post-translational modification by glycosylation in their extracellular domains. NRG1 is subject to modification in the region between the immunoglobulin and EGF domains by both N-linked and O-linked sugars [40]. NRG2 contains a potential site for addition of N-linked sugar in its extracellular domain but it is not known if this is modified [10]. NRG3 contains multiple sites for O-linked glycosylation but no sites for N-linked sugar addition [12] but it is not known if this modification occurs. NRG4 is glycosylated by at least one N-linked sugar [39]. It is interesting to note that the site in NRG2 and in NRG4 is in the EGF domain, but this does not appear to affect the latter's activity. Hayes et al. [39] show the position of the equivalent modification in the three dimensional structure of TGFα bound to the EGF receptor is unlikely to hinder binding. Whether this has any effect on the ligand in other respects is as yet unknown.

Functions of Non-classical Neuregulin Variants

As described above the various protein products of the four NRG genes which arise from alternative mRNA splicing are beginning to be catalogued. Their functions are however more elusive as, in addition to "conventional" products, partial products are produced which may be found in unexpected subcellular locations. The central paradigm of growth factor signalling in this family is that the EGF domain of the ligand interacts with the "extracellular domain" of the receptor (although this may possibly occur within cells as well as on the outer cell surface). However, in addition to gene products which encompass this 50 or so amino acid full EGF domain there are other products which contain only part of the ligand (for example in NRG1, NRG2 and NRG4 some variants, termed the γ-isoforms, consist of only the N-terminal two thirds containing four of the six cysteines which are involved in forming two disulphide bonds in the complete structure). We have even identified products of the *NRG3* gene which apparently contain no part of this region at all (Smart and Gullick, unpublished results).

We currently have no idea if the partial EGF domains can bind to their receptors. Molecular modelling of the equivalent N-terminal two thirds of TGFα bound to the EGF receptor suggests that they might, as the contacts between the first two thirds of the EGF domain and both the L1 and L2 domains of the receptor are preserved (Supplementary Figure 1) suggesting that such truncated ligands, if they were to fold and form the same disulphide bonds as in the complete structure could bind and stabilise the active receptor conformation. However their affinity and specificity may be affected since the "missing" final third of the structure is the part most buried in the contact surface (it is interesting that the final third is exactly, to the amino acid, that which is alternatively spliced in NRG1 and NRG2 to give the α and β variants [9]). Evidence against this occurring includes that numerous amino acid substitutions by site directed mutagenesis in this region prevent binding [41], removal of this region in Amphiregulin radically reduced binding affinity [42] and, finally, it is unlikely that these cytoplasmic forms will be in the oxidised (folded) state anyway.

What possible role there is for sequences lacking the EGF domain completely is entirely enigmatic.

Other domains in the extracellular region perhaps not surprisingly have been reported to have a role in modulating NRG activity mediated directly by its EGF-like region. Eto et al. [43] have reported that in *Xenopus laevis* an NRG-1 protein including the EGF-like domain and the upstream immunoglobulin had a thousand fold higher affinity for its receptor than the EGF domain alone. It will be interesting to obtain the three dimensional structure of this larger protein (particularly bound to its receptor) to see if there are any physical interactions between the domains or whether they co-operate independently in increasing receptor binding affinity. Wolpowitz et al. [44] have also disrupted the cysteine-rich domain found in the Type III variants of the *NRG1* gene in transgenic mice which resulted in abnormalities and death of multiple neural cell types and Schwann cells suggesting that these products have an important role in these cell types.

Other experiments have suggested that the intracellular regions of NRG1 may have a role in signalling (sometime known as "back signalling"). Liu et al. [45] created "knockout" mice in which the intracellular domain encoding exons of NRG1 were disrupted. In homozygote animals this led to death on day E10.5 from circulatory failure and abnormalities in cardiac and neural development. In support of these observations Bao et al. [46] have reported that binding of the extracellular EGF-like domain to a receptor stimulates proteolytic cleavage of the intracellular domain, which then translocates to the nucleus where it acts on regulators of apoptosis resulting in reduced neuronal cell death. It is not yet known if any similar activities lie in the cytoplasmic regions of NRG2, 3 or 4.

Not only is there much remaining to be learned about the structure and possible functions of these unusual splice variants but there are also mature products found in subcellular compartments where there may be no receptors (such as in the nucleoplasm) or where signalling has not previously been anticipated (such as in soluble forms in the cytoplasm). Neuregulins do not possess a classical signal or secretory sequence and yet are released into the extracellular space. This apparent paradox can readily be resolved by accepting that neuregulins are not secreted proteins in the sense that they are released as soluble forms by fusion of exocytotic vesicles with the plasma cell membrane. Instead those forms with a transmembrane sequence are incorporated into the endoplasmic reticulum membrane as transmembrane proteins which translocate to the Golgi where are they are glycosylated. They are then transferred to the plasma cell membrane by vesicles emanating from the Golgi apparatus. Here, at the cell surface, their release by proteolytic cleavage is tightly controlled by the activity of ADAM/TACE type proteases. Splice variants lacking the transmembrane sequence remain in the cytoplasm (such as the NRG4B forms), [26] or are transported to the nucleus (such as NRG1β3 via its nuclear localisation sequence), Golding et al. [47] (Fig. 2). For this system to work it is necessary that they do not have a signal/secretory sequence otherwise these patterns of localisation could not be achieved nor could their release by proteases be regulated. It has however been reported that ADAM 19 cleaves the ectodomain of the type I NRG1α1 in the Golgi apparatus [48] suggesting that regulation of release by proteolysis can occur prior to the proteins reaching the cell surface. Finally, in the MDA-MB-175 cell line, in which the *NRG1* gene is rearranged such that it acquires its N-terminal 560 amino acids from elsewhere, the fusion protein, which is a soluble β3 splice variant, is secreted [33]. In this apparently abnormal situation the fusion partner may provide a secretory signal so that the NRG1 protein evades the control of the protease system at the cell membrane and may be released freely after synthesis, potentially forming an unregulated autocrine loop. It is worth noting that in this case the system would be insensitive to inhibitors of the ADAM/TACE proteases (Fig. 2). Whether this is the mechanism that occurs generally in the 6% of breast cancers with rearrangements in NRG1 described below (and in the currently uncharacterised *NRG3* gene) remains to be determined.

The Role of Neuregulins 1–4 in Breast Cancer

The four NRG genes are each expressed at early stages in normal breast development [18] and NRG3 is crucial for

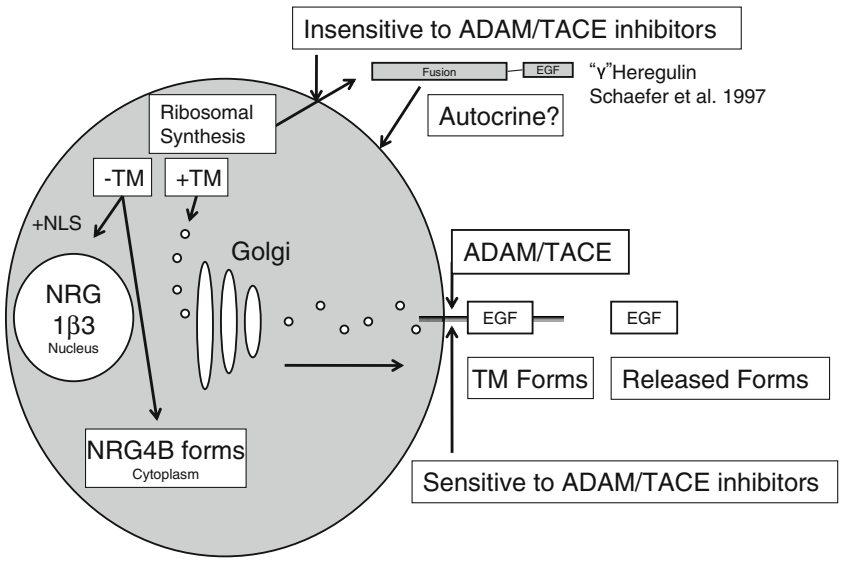

Figure 2 Biosynthetic pathways of NRG variants. After synthesis on ribosomes variants with a transmembrane region (+*TM*) are transported to the Golgi where they may be glycosylated. The products are then transported in vesicles to the plasma membrane where soluble products can be released by controlled proteolysis. Variants without a transmembrane sequence (−*TM*) are retained in the cytoplasm or, if they contain a nuclear localisation sequence (+*NLS*), they may be imported into the nucleus and localise in sub-nuclear compartments.

this process (reviewed by Howard in this volume). There is now also increasing evidence to suggest that the neuregulins may be important in breast cancer. *NRG1–4* genes are each expressed at high levels in a proportion of breast ductal carcinoma in situ [49] and in invasive breast cancers [25], but it is not known as yet which splice variants are involved.

The lack of knowledge of the properties of the NRG variants also makes it difficult to predict their roles. Moreover transgenic mice expressing NRG1 containing a full EGF domain in breast epithelial cells develop adenocarcinomas [50], yet addition of NRG1 to cells expressing one of their two direct receptors, HER4 results in differentiation or apoptosis [51]. The role of the more exotic splice variants is even more obscure although NRG1 and NRG3 are often found in the nuclei of breast DCIS cells [49] there is currently no model for their mechanism of action in this environment. It has been shown that the NRG1beta3 variant does not require any of the receptors in the family, or its EGF domain to reach the nucleus [47] but what it does when it gets there can only be a matter of speculation. It is interesting that two groups have reported that the NRG1-beta3 variant localises to intranuclear subcompartments identified as SC35 spliceosomes (also known as intrachromatin granules) and/or to nucleoli and have mapped the sub-nuclear localisation sequences responsible [47, 52]. Using two hybrid assays and pull down assays Breuleux et al. [52] have shown that the NRG1 interacts with a group of intranuclear proteins some of which are known to function in pre-mRNA splicing. It is currently under study as to whether NRG1 positively or negatively influences this activity. Clearly a great deal of experimental work needs to be done to address these several issues so that from this knowledge their actions in normal breast and in disease can be inferred.

There are however two observations derived from clinical material which can give us some clues. First, using immunohistochemical staining it has been shown that NRG 1–4 isoforms containing the EGF domain are expressed quite commonly at apparently elevated levels in DCIS [49] and breast cancer [25]. Second, the *NRG1* gene is rearranged in some breast (and pancreatic) cancer derived cell lines [53] and in about 6% of invasive breast cancers (19 of 323 cases) and making this, since breast cancer is so common, the most frequent consistent rearrangement in human cancer [54]. Preliminary data also suggest the *NRG3* gene is rearranged in some breast cancers (Paul Edwards, personal communication). While the nature of the rearrangements has been very carefully studied [55] the effect on expression levels or splicing is not yet known. These smoking guns suggest that aberrant expression may be influencing the cells along the path to transformation. There are however relatively few studies on NRGs in clinical material and none so far with substantial numbers of patients and so their associations (if any) with molecular or clinical parameters including relapse or death are unexplored. Raj et al. [56] have reported that low levels of NRG1alpha were associated with poor prognosis but the effect of the other products of this and the other genes remain to be explored. This deficiency can however be easily rectified now that antibodies which work in fixed tissue are available [25, 26]. Then using PCR or splice variant specific antibodies these relationships can be narrowed down to observations on particular gene products.

Several recent studies suggest that the neuregulins and their receptors will be of more than academic interest, not least as the other interacting receptors in the family, the EGF receptor and HER2 proteins, are the targets for many signal transduction inhibitor drugs. Recent work by Zhou et al [31] has shown that autocrine activation of the HER3 receptor by neuregulin/heregulin is responsible for resistance to gefitinib treatment in some non-small cell cancer cell lines. Other work has shown increased expression and activation of HER3 and activation of the downstream PI3kinase/Akt pathway can be responsible for resistance to small molecule receptor tyrosine kinase inhibitors [57] and that loss of PTEN which regulates this latter pathway is a major factor in resistance to trastuzumab (Herceptin) in breast cancer [58–60]. Ritter et al. [61] have also shown that cell lines selected to be resistant to Trastuzumab have up-regulated the EGF receptor and several ligands of the family including neuregulin/heregulin. Conversely, transfection of MCF-7 cells, which express quite low, normal, levels of HER2 with an isoform of NRG1 rendered them sensitive to treatment with trastuzumab in vivo [62]. Patients with low levels of HER2 but high levels of NRG1 treated with trastuzumab had longer relapse free interval and overall survival than patients with low levels of both, from which the authors suggest that there may be another group of breast cancer patients who may gain benefit from the drug [62].

Thus there is considerable interest in inhibiting these pathways directly by antibodies, such as to the extracellular domain of the HER3 receptor [63] or small molecules interfering with components of the PI3kinase/Akt pathway or indirectly by using compounds that inhibit the ADAM proteases responsible for the release of active neuregulins from the cell surface [31, 64]. It should however be noted that activation of HER3 may occur by other mechanisms such as increased expression and activity of the Met receptor [65] and so the use of particular inhibitors may be predicated on the type of over-activity in the system. Finally it has been shown that NRG1alpha is involved as a motility factor in Paget's disease where, in most cases HER2 is highly overexpressed [66] possibly suggesting a potential target for prevention of metastatic spread.

In summary, there are so many exciting observations already on the NRG family that, although their complexity may be somewhat daunting, there will be many more to come. Some of the knowledge gained should be of use in understanding how these pathways are altered in disease and how signal transduction inhibitor drugs can be best designed and evaluated.

Concluding Remarks

The four neuregulin genes have multiple controls over the place, time and extent of their expression, a complexity which is multiplied many fold by alternative splicing proteolytic processing, sub-cellular localisation and degradation of their products. There are established receptor-mediated models of action of forms which contain a complete EGF domain but as yet there is not for the γ class of products which contain only the first two thirds of this region. Evidence is growing that even more enigmatic variants are produced by splicing or proteolysis which have no EGF domain at all.

It is clear that as a whole this ensemble of molecules have multiple roles in normal tissues and in disease. While it looks currently a challenging task to unravel this network it may none the less be rewarding giving insights into the biology of multi-cellular organisms and may have profound effects on how we diagnose and treat several common human conditions.

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