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HER3 comes of age; New insights into its functions and role in signaling, tumor biology, and cancer therapy

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Abstract

The human epidermal growth family (HER) of tyrosine kinase receptors underly the pathogenesis of many types of human cancer. The oncogenic functions of three of the HER proteins can be unleashed through amplification, overexpression, or mutational activation. This has formed the basis for the development of clinically active targeted therapies. However, the third member HER3 is catalytically inactive, not found to be mutated or amplified in cancers, and its role and functions have remained shrouded in mystery. Recent evidence derived primarily from experimental models now appear to implicate HER3 in the pathogenesis of several types of cancer. Furthermore, the failure to recognize the central role of HER3 appears to underly resistance to EGFR or HER2 targeted therapies in some cancers. Structural and biochemical studies have now greatly enhanced our understanding of signaling in the HER family and revealed the previously unrecognized activating functions embodied in the catalytically impaired kinase domain of HER3. This renewed interest and mechanistic basis has fueled the development of new classes of HER3-targeting agents for cancer therapy. However, identifying HER3-dependent tumors presents a formidable challenge and the success of HER3-targeting approaches depend entirely on the development and power of predictive tools.

Background

The human epidermal growth factor receptor (HER) family of receptor tyrosine kinases (RTKs) are the most extensively studied family of RTKs and strongly implicated in the pathogenesis of many types of human cancer. The family includes the four highly homologous members EGFR (HER1), HER2 (ErbB2), HER3 (ErbB3), and HER4 (ErbB4), sharing a structure that consists of a ligand-binding extracellular domain (ECD), an intracellular kinase domain (KD), and a c-terminal signaling tail. Signaling is mediated through ligand-induced receptor dimerization and transphosphorylation, leading to activation of cytoplasmic signaling pathways. The individual HER proteins have non-redundant functions and unique attributes and heterodimerization constitutes the predominant mode of signaling in this family. The functions of the third member HER3 have been least understood, and mounting evidence implicating it in human cancer pathogenesis have deepened interest in resolving the mysteries surrounding it.

The unique attribute that separates HER3 from the other HER proteins is its evolutionary divergence at critical residues within the KD, locking it in the inactive conformation, thus devoid of catalytic kinase activity (1-3). Our traditional models of RTK function have not been able to deal with this finding, and by default, HER3 has been considered to function merely as

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a signaling substrate for other HER proteins, analogous to the functions of IRS1 and IRS2 with the insulin receptor. This model would appear to dismiss functionality within the HER3 KD, a concept that is difficult to reconcile in evolutionary terms. The recent landmark study revealing a highly unique mechanism underlying KD activation in the HER family now finally identifies the potential functions of a catalytically inactive KD such as that of HER3. In the HER family, dimerization of the KDs occurs in an asymmetric configuration leading to the allosteric activation of one KD by the other (4). In this interaction the “activator” KD has no catalytic role, immediately suggesting that the HER3 KD may be a highly specialized allosteric activator of its HER family partners (figure 1a). The functions of the HER3 KD as a specialized allosteric activator have been confirmed in biochemical assays (3).

In contrast to the other HER proteins, HER3 is not transforming when overexpressed or constitutively activated by continuous ligand stimulation (5) and there are currently no mutational alterations known to confer oncogenic activities to HER3. This may be due to a number of mechanisms in place that appear to function to restrain the signaling functions of HER3 (figure 1b). The absence of catalytic activity is only one such mechanism. In the absence of ligand activation, the HER3 c-terminal tail binds and covers its activation surface *in trans*, restraining its allosteric activation functions (3). In addition, the clustering of HER3, based on both ECD interactions and ICD interactions, appears to provide yet additional restraint through its sequestration away from EGFR and HER2 (3,6). HER4 may also participate in HER3 sequestration, providing some insight into why increased HER4 expression has been found to be associated with a less malignant biology in breast cancers (3). Additionally, the Ebp-1 protein has been known to interact with a region of HER3 that was recently identified as the dimer-stabilizing juxtamembrane region (7,8). As such, Ebp-1 binding may potentially function to prevent premature dimerization, providing yet another mechanism nature has provided to restrain HER3 from inappropriately activating its HER partners.

When the restraints on HER3 are lifted, HER3 functions not only as a specialized allosteric activator of other HER proteins, but also as their signaling substrate. The 14 tyrosines in the c-terminal signaling tail of HER3, when phosphorylated, can potentially dock numerous SH2 or PTB binding proteins involved in a number of different intracellular signaling pathways (figure 1c). Whether all of these tyrosines are phosphorylated in cells, and whether all of the described interactions are physiologically relevant remain to be defined. But the one critically important and well established signaling activity of HER3 is its unique and potent ability to activate downstream PI3K and Akt pathway signaling by virtue of six consensus phosphotyrosine sites, not present on EGFR or HER2, which bind the SH2 domain of the three regulatory subunits of PI3K (9-11)(figure 1c). Activated PI3K phosphorylates membrane phosphoinositides, leading to recruitment and activation of PDK1 and Akt. Akt lies at the hub of a plethora of downstream pathways, in particular in an intricate upstream and downstream relationship with two mTor-containing complexes, and is in a position to control many biological processes critical for tumorigenesis including translation, survival, nutrient sensing, metabolic regulation, and cell cycle control (12)(figure 1a).

The link between HER3 and the Akt pathway not only confers oncogenic capabilities to its kinase-active HER family partners, but provides a signaling node that can potentially be exploited by other signaling pathways to engage the activities of Akt. Most tumors require PI3K/Akt signaling for their survival, and this is often achieved by upstream receptor tyrosine overactivity, by mutational activation of PI3K or inactivation of PTEN. However the induction of HER3 provides another pathway towards this end, and indeed the expression of HER3 appears to be more dynamic than other HER proteins and inducible when required. The finding that the induction of HER3 expression or signaling is associated with drug resistance in several cancer models supports such a role. HER3 expression or signaling is associated with resistance to HER2 inhibitors in HER2-amplified breast cancers (13), with EGFR inhibitors in lung

cancers (14), with pertuzumab resistance in ovarian cancers (15), with anti-estrogen therapies in ER-positive breast cancers (16-19), with EGFR inhibitors in head & neck cancers (20), with hormone resistance in prostate cancers (21), and with IGF1R inhibitors in hepatomas (22). In most of these scenarios, it is assumed that HER3 phosphorylation is driven by one of its HER family kinase partners. A more promiscuous role for HER3 as a substrate of other kinases is possible, and at least suggested by the c-MET induced activation of HER3 signaling (14), however this has not been directly demonstrated.

The cellular signals that regulate HER3 expression are only beginning to be identified. HER3 protein expression is regulated by both transcriptional and post-transcriptional mechanisms. Post-transcriptionally, it is regulated by the E3 ligase Nrdp1 and the Nrdp1 regulator USP8 (23). USP8 in turn is regulated by Akt and this pathway potentially links HER3 expression with downstream Akt activity constituting the potential reciprocal regulation of HER3 expression with Akt activity. The transmembrane protein LRIG1 is another negative regulator of growth factor receptor signaling. It associates with all four HER family members, although current studies have only addressed its role in the specific regulation of EGFR and HER2 (24-26). The transmembrane mucin MUC4 alters HER2 and HER3 trafficking, stabilizes their membrane localization, consequently increasing their signaling functions (27). HER3 is also regulated transcriptionally, as seen in response to TKI treatment of HER2-amplified breast cancer cells ((13) and manuscript in submission). The expression of HER3 is also under regulation by several miRNAs identified thus far, including miR205, miR125a, and miR125b (28,29).

The precise functional relevance of HER3 across the spectrum of human cancers remains largely unknown at this time. There is solid mechanistic and experimental evidence supporting its tumor promoting functions in subsets of breast and lung cancers, as well as a body of more speculative, descriptive, and sometimes conflicting data in many other cancers. The existing literature regarding the expression and relevance of HER3 in human cancers is summarized in Table 1. The data sets vary considerably in their methodologies and can not be easily compared. While some investigators analyze expression, others analyze over-expression relative to normal tissues or within the sample sets. There is also great variance in the reported localization of HER3, including nuclear expression on IHC studies, and these should be interpreted with caution until confirmed by the appropriate biochemical studies.

Experimental evidence has solidly established the critical role of HER3 as a co-receptor for the amplified HER2 oncogene, most commonly seen in breast cancers, but also in a variety of other tumor types. Although HER3 is not transforming by itself, it is synergistically co-transforming with HER2 (30). In fact, the expression of HER3 is rate-limiting for HER2-induced transformed growth and if HER3 expression is knocked down, HER2-amplified tumors cease to grow and undergo apoptotic cell death (31,32). Considering that HER2 expression is generally a constant in this tumor type, the regulated expression of HER3 functions as a volume control for HER2-HER3 signaling. This rheostat function is exemplified during attempts to inhibit HER2-HER3 signaling with tyrosine kinase inhibitors (TKIs). Treatment of HER2-amplified breast cancers with HER2-targeting TKIs leads to a rapid compensatory increase in HER3 expression, localization, and signaling activity, revealing a significant reserve capacity embodied within the HER2-HER3 signaling complex that renders it highly resilient to anti-cancer therapies (13). In this context, HER3 is both the allosteric activator of HER2 kinase as well as its critical signaling substrate, and as such functions both upstream and downstream of HER2, redefining the HER2-HER3 complex as the functionally relevant oncogenic driver of the disease.

Other lines of evidence also demonstrate an important role for HER3 as a co-receptor for EGFR in a subset of lung cancers. In a comparison of lung cancer cell lines that are sensitive or resistant

to the EGFR TKI gefitinib, the best marker of sensitivity to gefitinib appears to be the HER3-dependent activation of PI3K (33). This EGFR-HER3 interdependency is seen in tumor cell types harboring mutationally activated EGFR as well as wild-type EGFR, revealing a central role for EGFR-HER3 signaling in this disease. Treatment of patients with gefitinib is highly effective in these patients, although the development of acquired resistance is universal. While resistance typically develops as a result of secondary mutational events within the EGFR kinase domain rendering EGFR drug-resistant, rarely resistance can also develop through overactivity of the cooperating proto-oncogene c-MET and the c-MET-dependent phosphorylation of HER3, further highlighting the central role of HER3 in the pathogenesis of this disease (14).

There is a mounting body of evidence that implicates HER3 and the HER family in the pathogenesis of melanoma. HER3 is not expressed in normal melanocytes, but HER3 expression is seen in many malignant melanomas and is associated with more advanced stage, increased proliferation, and decreased survival (34,35). This suggests that HER3 may be important in melanoma progression, and experimental models of HER3 knockdown suggest a role in migration and invasion (35,36). The HER kinase partner for HER3 in melanomas is not well identified at this time. HER2 is not commonly expressed in melanomas, but EGFR is widely expressed and HER4 is mutationally activated in a subset of melanomas (36-39). The evidence that HER3 may be functionally important in melanoma is mounting, but much more work needs to be done to confirm and define its role.

EGFR targeting therapies show clinical activity in colon cancers, but the mechanistic basis for this activity remains undefined. Several studies looking at the expression of HER family members have produced largely conflicting and overall inconclusive results. However a recent mouse conditional model reveals that loss of HER3 in the intestinal epithelium leads to the concomitant loss of HER4 and prevention of tumorigenesis in ApcMin mice (40). These results were also reproduced in human colon cancer cells and will undoubtedly lead to much further analysis of colon cancers, focusing on HER3 and HER4. HER3 expression in ovarian cancers is associated with decreased survival and with resistance to the HER2-targeting mAb pertuzumab (15,41), suggesting a functionally relevant role for HER3 in a subset of ovarian cancers. HER3 expression is upregulated in clear cell sarcomas of soft tissue and the receptor activated by autocrine neuregulin expression in many of them (42,43).

Clinical Translational Advances

The important role of HER3 as a signaling hub for the HER3 family has identified it as a candidate target for drug discovery and numerous HER3-targeting programs are currently underway to explore the potential of this new target. But HER3-targeting initiatives are faced with some unique challenges, both in the technical and strategic realms.

A principal technical challenge concerns what functionality of HER3 to target. Unlike the other HER family members, the functions of HER3 are not mediated through enzymatic catalytic activity and at this point, it does not seem that the ATP analog class of tyrosine kinase inhibitors would be suitable for this target. Whether ATP binding is required or plays a role in the stimulatory function or stability of the HER3 KD remains to be determined. If ATP is indeed found to be required for this function, this could lead to the development of ATP analog drugs that target non-catalytic functions, sometimes referred to as “pseudokinase” inhibitors. The stimulatory functions of the HER3 KD can also be targeted with newer classes of allosteric inhibitors. These could potentially target the KD dimerization interface, preventing the allosteric activation of EGFR or HER2 by HER3, or they could target the juxtamembrane latch, destabilizing the dimerized conformation of the HER3 KD. Much of the functions of the HER3 KD are still shrouded in mystery and the endeavor to target the functions of the HER3 KD require exploratory and potentially pioneering work into uncharted waters, and their products

would be first-in-class molecules. Although risky as investments, such class inventions can lead to advances in targeted therapies that extend far beyond the realm of HER3.

Targeting the extracellular domain of HER3 with macromolecules such as antibodies is much more amenable to existing well established pharmaceutical platforms and several such programs are currently underway. Inherent in this approach are also some uncertainties regarding the optimal activity to be targeted by antibodies. The most traditional approach with transmembrane receptors has been to target a region that interferes with ligand binding and ligand-induced activation. The success of such a treatment hypothesis depends on an underlying assumption that it is the ligand-activated conformation of HER3 that is the driving force in tumor progression. The validity of this assumption is not at all certain and may indeed vary among different cancer subtypes and even among individual patients tumors. The anti-HER2 monoclonal antibody pertuzumab was developed to target the dimerization interface of HER2 and disrupt ligand-induced HER2-HER3 dimerization and signaling. While pertuzumab appears to be quite effective at inhibiting neuregulin-induced HER3 signaling (32,44), it appears to be much less effective at disrupting the elevated basal state of ligand-independent HER2-HER3 interaction and signaling in HER2-overexpressing tumor cells (45,46). This is not surprising as liganded and unliganded conformations of the HER3 ECD have very different structural characteristics. The nature of the physical interaction that underlies HER2-HER3 transactivation in the absence of ligand are not well understood, and at this point “ligand-independent signaling” remains a concept without a structural descriptor, although several hypotheses regarding this interaction can be proposed.

The major strategic challenge in the development of HER3-targeting agents is predicting patient populations and disease subtypes that would be amenable to this treatment modality. Unlike other targets, the tumor-promoting functions of HER3 are not harnessed through its over-expression, amplification, or mutation, making it more difficult to identify HER3-dependent tumors. At this point, a critical function for HER3 has been confirmed in HER2-amplified breast cancers, and strongly implicated in a subset of EGFR-driven lung cancers. The best marker to identify the functional relevance of HER3 in other tumors is the detection of phosphorylated HER3, which is the principal reporter of HER3 signaling. Numerous antibody reagents are available that detect phosphorylated HER3 tyrosine residues on western blots, however a reagent that can reliably detect p-HER3 in formalin-fixed paraffin-embedded tissues has been a challenge to develop. There are limited published reports containing p-HER3 immunostaining data using available commercial reagents (34). However in our hands, these same reagents fail the test of p-HER3 specificity when assayed using positive (ligand-stimulated) and negative (TKI-treated) control cell lines which are fixed in formalin and embedded in paraffin. The success of HER3-targeting therapies is entirely dependent on the validity of the p-HER3 biomarker assays and the validity and reliability of such assays is of utmost importance.

Another potential marker of HER3 signaling activity is the expression of its ligands in the tumor microenvironment. However this requires highly sensitive validated antibody reagents with specificity controls capable of detecting very dilute concentrations of the target ligands within the extracellular matrix. While this may be implausible, as a surrogate, most studies have attempted to indirectly assay ligand activity by immunostaining their transmembrane precursors in tumor cells. This approach discounts the role of stromal derived ligands, as well as proteolytic cleavage as a limiting and regulatory step in ligand release, while also appealing to the ongoing controversial proposition that membrane-bound ligand is competent and active at signaling. Most of these studies report frequent expression of neuregulins in tumors and were reviewed recently in the Molecular Pathways series (47). The data sets vary considerably with regards to membrane, cytoplasmic, nuclear, or stromal expression, and the true

specificities of the signals are difficult to know, specially in light of universal expression reported by some studies.

While structural, biological, and clinical studies are beginning to unmask the role of HER3 signaling in human cancers, our understanding of this receptor is still in its infancy. Ongoing experimental studies and the arrival of HER3-targeting agents will provide much more insights into the relevance and functions of this receptor.

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Figure 1a.

Schematic representation of HER3 engaged in dimerization and actively signaling. The dimer is reinforced by interaction of the juxta-membrane segments, forming a stabilizing latch. Through its activating interface, HER3 engages and allosterically activates its kinase partners, in this case HER2. Phosphorylation of its c-terminal tail leads to recruitment of adapter proteins leading to activation of Ras and PI3K. Recruitment and activation of PI3K leads to phosphorylation of membrane phosphoinositides producing PIP3, which in turn docks the PH domain-containing proteins PDK1 and Akt. Membrane-bound Akt is phosphorylated and activated by PDK1 and Tor-complex 2 (TORC2). Activated Akt proceeds to phosphorylate a plethora of cellular substrates involved in diverse biological processes.

Figure 1b.

Schematic representation of mechanisms in place that restrain HER3 from signaling. If not transcriptionally, post-transcriptionally, or post-translationally repressed, much of HER3 is sequestered into clusters mediated through N-lobe head-to-head interactions and reciprocal c-tail interactions (3). The proximal c-terminal tails bind and cover the activating interface of the KD, obstructing its allosteric activating function. Even when not sequestered, HER3 is incapable of self-activation as it lacks the receiving interface for allosteric activation and its active site is permanently locked in the inactive state. It can only engage as the activating partner with the other HER family members, as shown in 1a.

Figure 1c.

Schematic depiction of the 14 tyrosines on the c-terminal signaling tail of HER3. Whether or not all of these tyrosines undergo phosphorylation in cells has not been confirmed. But phospho-peptide binding studies have identified many SH2 and PTB domains that are capable of high affinity binding to these phosphotyrosine sites as indicated. Only binding activities with nanomolar range *K_d* are shown here. The detailed binding data are available from the data sources (48,49). The six PI3K binding sites are confirmed to undergo phosphorylation and can mediate the interaction with each of the three regulatory subunits of PI3K. Additional tyrosines within the kinase domain show binding activity in phosphopeptide assays, but are not shown here.

Material	Ref	N	Assay Type	Expressed or overexpressed	Amp/Mutation	Staining Pattern	Correlations / comments
Pancreatic Cancer							
Pancreatic cancers	(50)	141	IHC	(35%) 49/141	N/A	C, N	No correlation with tumor grade, stage, or histological type
Pancreatic cancers	(51)	27	NB	63% (17/27)	None by SB	N/A	
		58	IHC	47% (27/58)	N/A	N/A	HER3 expression correlated with more advanced tumor stage/shorter postoperative survival; no correlation with histological grade
Pancreatic cancer cell lines	(51)	6	NB	67% (4/6)	N/A	N/A	
Pancreatic cancer cell lines	(50)	11		73% (8/11)	None by SB	N/A	
Colorectal Cancer							
Colon Ca primary tumor	(52)	30	NB	(53%) 16/30	NS	N/A	
Colon Ca liver metastases	(52)	34	NB	56% (19/34)	N/A	N/A	
Colon cancer cell lines	(52)	8	NB	50% (4/8)	N/A	N/A	
Colorectal cancers	(53)	109	IHC	70% (76/109)	N/A	C	HER3 correlated with better patient outcome
							HER3 correlated with lymphoinvasion
Colorectal cancers	(54)	55	IHC	78% (43/55)	N/A	C	HER3 correlated with decreased survival but not tumor size or grade
Colorectal cancers	(55)	109	IHC	28% (30/106) 9% (10/106) very high	N/A	N/A	No correlation with disease free survival
Colorectal cancers	(56)	35	IHC	89% (31/35)	N/A	C, M	HER3 overexpression correlated with HER2 overexpression
Colorectal cancers	(56)	35	NB	64%	None by SB	N/A	No correlation with tumour localization, differentiation; HER3 expression associated with lower age
Colorectal cancers	(57)	125	IHC	3% (3/125)	C, M	N/A	HER3 inversely associated with tumor stage; no association with other clinicopathological indicators
Colorectal cancers	(58)	16	IHC	69% (11/16)	N/A	C	

Material	Ref	N	Assay Type	Expressed or overexpressed	Amp/Mutation	Staining Pattern	Correlations / comments
Gastric Cancer							
Gastric cancer	(58)	17	IHC	70% (12/17)	N/A	C & M	
Gastric cancers	(59)	102	IHC	14% (14/102)	N/A	C & M	HER3 correlated with advanced stage and worse prognosis.
Esophageal cancer	(58)	26	IHC	100% (26/26)	N/A	Some M	
Lung Cancer							
Pulmonary Carcinoid	(60)	24	IHC	100% moderate-intense	N/A	C in 100%	
Adenocarcinoma	(61)	39	IHC	46% (18/39)	N/A		
NSCLC	(62)	42	IHC	19% (8/42)	N/A	N/A	Increased HER3 expression correlated with response to TKI
NSCLC cell lines	(62)	6	WB	67% (4/6)	N/A	N/A	Increased HER3 expression correlated with response to TKI
NSCLC	(63)	441	IHC	18% (82/441)	N/A	C, M	Increased HER3 expression correlated with shorter survival
NSCLC cell lines	(33)	8	WB	37.5% (3/8)	N/A	N/A	No association with clinical features, prognosis or DFS
NSCLC	(64)	91	q-PCR microarray	NS	N/A	N/A	
NSCLC cell lines	(64)	21	FISH		80% (16/20)	N/A	Low grade amplifications (> 3 copies/cell)
Melanoma							
Primary melanomas	(35)	130	IHC	65% (85/130)	N/A	M	High HER3 expression correlates cell proliferation, decreased survival; No association with clinical features
Melanoma metastases	(35)	87	IHC	40% (35/87)	N/A	M	
Melanocytes and melanoma cells lines	(65)	4	WB	0	N/A		Melanoma cells have less HER3 expression than melanocytes
Clear Cell Sarcoma of Soft Tissue (CCSST)							
CCSST cell lines	(42)	8	WB, CGH	100% (8/8)	none	N/A	

Material	Ref	N	Assay Type	Expressed or overexpressed	Amp/Mutation	Staining Pattern	Correlations / comments
CCSST	(43)	20	IHC	90% (18/20)	N/A	N/A	
CCSST cell lines	(43)	6	NB, WB	83% (5/6)	N/A	N/A	
Ovarian Cancer							
Epithelial Ovarian Cancer	(66)	46	IHC	41% (19/46)	N/A	C	No association with age tumor stage, differentiation, ploidy, cell cycle
Endometrioid carcinoma of the ovary	(67)	28	IHC	50% (14/28)	N/A	N/A	p53 and HER3 overexpression correlated with worse survival
Granulosa cell Tumors	(68)	12	IHC	16.7% (2/12)	N/A	C	
Epithelial Ovarian Cancer	(69)	56	IHC	73% (41/56)	N/A	N/A	No correlation with overall survival or disease correlation
Epithelial Ovarian Cancer	(70)	103	IHC	3% (3/103)	N/A	N/A	
Epithelial Ovarian Cancer	(41)	116	IHC	53.4% (62/116)	N/A	C	HER3 expression associated with decreased survival, histologic grade and type, residual disease, age
Ovarian cancers	(71)	101	ELISA q-PCR	higher mean value	N/A	N/A	HER3 expression levels increased with age; no correlation with clinicopathological characteristics
Prostate Cancer							
Prostate cancers	(72)	143	IHC	none	N/A	C, N	No correlation with histopathologic grade
Prostate cancers	(73)	81	Tissue Array IHC	none	N/A	C, N	Nuclear staining was significantly higher in tumor vs. normal tissue No correlation with histopathologic grade
Breast Cancer							
Ductal carcinoma in situ	(74)	57	IHC	35% (20/57)	N/A	C= 16 cases M= 4 cases	HER3 expression correlates with HER2 over-expression; but HER3 over-expression correlates with lack of HER2 overexpression
breast cancers	(75)	97	IHC	28.8%	N/A		trend towards node negative and better survival

Material	Ref	N	Assay Type	Expressed or overexpressed	Amp/Mutation	Staining Pattern	Correlations / comments
breast cancers	(76)	195	IHC	22%	N/A	C	Overexpression associated with lymph node metastases, but not survival
breast cancers – node negative	(77)	212	IHC	65% (138/212) present 13% (28/212) strong	N/A	C = 138 cases M = 28 cases	No association with relapse or DFS; no association with clinicopathological features
breast cancers	(78)	268	RT-PCR				higher HER3 associated with better survival
breast cancers	(79)	95	IHC	53% (50/95)	N/A	C	associated with higher grade
breast cancers	(80)	220	IHC	17.5%	N/A		No association with grade
breast cancers	(81)	278	FISH		Some with low grade <2.3X	N/A	Low grade HER3 amplification negative effect on DFS
breast cancers	(81)	173	IHC	75% positive	N/A		No correlation with DFS

WB = western blot, NB = northern blot, SB = southern blot, IHC = immunohistochemistry, CGH = comparative genomic hybridization, FISH = fluorescent in situ hybridization, qPCR = quantitative PCR, N/A = not available; NS = not stated; DFS = disease free survival

CCSST = Clear Cell Sarcoma of Soft Tissue, NSCLC = non-small cell lung carcinoma

Analyses were performed on primary patient tumor samples unless otherwise stated