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REVIEW

Cytoplasmic Overexpression of HER2: a Key Factor in Colorectal Cancer

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Abstract: Trastuzumab, a humanized mouse monoclonal antibody directed against HER2 (Human Epidermal Growth Factor Receptor 2), is currently a keystone in the treatment of breast cancer. Meanwhile, trastuzumab has been validated for use in other types of cancer too. But the data on HER2 expression in colorectal cancer are ambiguous, with reported overexpression of HER2 varying between zero and 84%. In this review these studies are evaluated and compared. It shows that many factors influence the determination of HER2-expression, especially of the intracellular fraction of HER2. It is concluded that although membranous overexpression of HER2 is low in colorectal cancer with only 5% of all patients being positive, a significant proportion of the patients (30%) shows cytoplasmic HER2 overexpression. The clinical impact of enhanced intracellular HER2 is not known, because the nature and origin have not completely been revealed yet. Enlightening this process could be a stepping stone towards targeting of intracellular HER2 as a treatment option.

Keywords: HER2, ErbB2, Her2/neu, colorectal cancer, survival, membranous, cytoplasmic, immunohistochemistry, tissue microarray, biomarker, trastuzumab, herceptin

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Background

Colorectal cancer is a major worldwide health problem with an annual incidence of 1.2 million and an annual mortality of over 600,000 people.[1,2] One of the reasons for the relatively high mortality is high tumor stage at the time of diagnosis, caused by the lack of symptoms or the presence of aspecific symptoms during the initial stages of disease. With an increase in tumor stage, the risk of recurrence after resection rises and the chances of survival deteriorate. Chemotherapy has shown to be an efficient strategy for adjuvant therapy, but is still not capable of preventing recurrence in all patients.[3] Therefore there is numerous ongoing research for alternative compounds to be used as adjuvant therapy. Monoclonal antibodies and other biologicals, targeting tumor-associated proteins and blocking essential processes of the tumor, are extensively studied. A crucial step in this process is the identification of tumor specific proteins that can be targeted by these compounds. One of these targets is HER2, which is primarily associated with breast cancer. This review provides an overview of all accessible studies that reported on HER2 overexpression in colorectal cancer, focusing especially on the differences between these studies.

HER2

Over the last two decades, the transmembrane tyrosine kinase receptor Human Epidermal Growth Factor Receptor 2 (HER2, also known as HER2/neu, ErbB2 or p185) has been shown to be an effective target for adjuvant therapy for especially breast cancer. HER2 is found to be upregulated in 20%–30% of all breast cancers. The 40–100 fold protein upregulation is due to a genetic amplification to 25–50 copies.[4] Slamon et al showed that tumors with overexpression of HER2 are more aggressive and, consequently, these patients have a worse prognosis compared to patients with HER2-negative tumors.[5,6] Trastuzumab (Herceptin®), a monoclonal antibody against the extracellular domain of HER2, showed a dramatic improvement in survival as well as recurrence rate when used as adjuvant therapy after breast cancer surgery. The recurrence risk dropped by 40% and the mortality rate by 30%.[7,8] This novel HER2-targeted therapy required a standardized scoring of immunohistochemical HER2 tumor staining to select the breast cancer patients that could benefit from trastuzumab. The scoring of HER2-status comprises four different outcomes: 0 (no staining or staining in <10% of the tumor cells), 1+ (a faint or incomplete membrane staining in >10% of the tumor cells), 2+ (a weak to moderate complete membrane staining in >10% of the tumor cells) and 3+ (an intense and complete membrane staining in >30% of the tumor cells).[9] There are multiple immunohistochemical kits available for HER2-staining, but the polyclonal antibody-based Herceptest (Dako, Glostrup, Denmark), which has been approved by the FDA (US Food and Drug Administration), is mostly used. Remarkably, it is reported that monoclonal antibody-based kits have a better agreement with HER2 gene amplification, as determined by FISH (fluorescence in situ hybridization).[10,11] Generally, the 3+ cases have an excellent concordance with FISH, while the 2+ results are more equivocal.[12] Current guidelines demand a FISH-analysis to determine the gene amplification in 2+ cases.[13]

Colorectal HER2 expression

After the breakthrough in breast cancer, HER2 has been evaluated as a target in other tumor types. After a 37% improvement in overall survival, this recently led to FDA-approval for the use of trastuzumab in HER2-positive metastatic gastric cancer.[14]

Several studies evaluating HER2 in colorectal cancer resulted in a large debate at the end of the last century because overexpression rates varied between zero and 84%.[15] Also the clinical significance of HER2 in these publications was not consistent, with some publications associating HER2 overexpression to survival,[16–18] while others failed to find such a correlation.[19–21] Most researchers agreed that the differences in expression were likely due to differences in technical approaches, antibodies, and scoring protocols, but conclusive data were never presented. Since then a large number of immunohistochemical studies have emerged about HER2 in colorectal cancer. The majority showed membranous overexpression rates between zero and 15%.[22–31] Remarkably, some studies reported both membranous and cytoplasmic overexpression with much higher rates up to 60%.[32–38] Up to now, there is no consistency about the proportion of HER2-overexpression in colorectal tumors.

Immunohistochemical detection of HER2

The major difficulty in comparing immunohistochemical studies is the variety in antibodies. Additionally, differences in technical procedures such as tissue fixation, slide storage procedures, antigen retrieval, and incubation time could influence the staining outcome. The type of HER2-antibody used varied widely in the 27 analyzed studies in this review. In total, 15 different types of antibodies were reported, eight monoclonals and seven polyclonals (Table 1). The claim of Arnaout et al in an early study that polyclonal antibodies mainly stain cytoplasmic HER2, in contrast to monoclonal antibodies staining primarily membranous HER2, was not confirmed in more recent publications.[41] For example, Half et al observed a cytoplasmic staining in 64% of the cases using monoclonal antibodies CB11 and 3B5.[23] Furthermore, there is evidence that some antibodies might not be completely specific for HER2, which could result in aberrant staining patterns. Schrohl et al showed that monoclonal antibody clones 4B5 and CB11 (respectively from PATHWAY®; Ventana Medical Systems Inc, Tucson, AZ, USA and Oracle®; Leica Microsystems GmbH, Wetzlar, Germany) cross-reacted with HER4. The antibodies used in the much used Herceptest, did not cross-react with any other HER-receptors.[47]

Although differences in antigen specificity are presumably the main cause for the differences between various reports, some studies using the same antibody still obtained varying results. For example, antibody NCL-CB11 (Novocastra Laboratories, Newcastle Upon Tyne, UK) is used in 5 different studies with a reported overexpression rate between 22% and 82%. Therefore, procedural variations might also substantially contribute to the variations found

Table 1. An overview of the antibodies used in the 27 studies analyzing HER2 overexpression in colorectal cancer. The antibodies are organized based on the scoring categories of their publications: studies that split into membranous overexpression and cytoplasmic overexpression (A), and studies without distinction between the overexpressing regions (B).

(A) Membrane and cytoplasm HER2 expression evaluated independently

Antibody	Company	Monoclonal/ polyclonal	Patients (n)	% membrane	% cytoplasmic
4D5[41]	Genentech	Monoclonal	70	0.00	0.00
HER14[41]	Genentech	Polyclonal	70	0.00	34.29
21N[41,42]		Polyclonal	93	1.08	5.38
NCL-CB11[16,22,26]	Novocastra	Monoclonal	310	2.58	30.97
No name[18,29]	Nichirei	Polyclonal	390	2.82	24.87
e2-4001[23]	Neomarkers	Monoclonal	96	5.21	63.54
3B5[23]	Oncogene science	Monoclonal	96	5.21	63.54
Herceptest[25,27,28,30,31]	Dako	Polyclonal	857	7.47	
4B5[24]	Ventana	Monoclonal	106	10.38	
CB11[43]	Biogenex	Monoclonal	86	13.95	
Z4881[36]	Zymed	Polyclonal	137	47.45	
A0485[34]	Dako	Polyclonal	74	51.35	

(B) Membrane and cytoplasm evaluated together

Antibody	Company	Monoclonal/ polyclonal	Patients (n)	% positive staining
e2-4001[38]	Neomarkers	Monoclonal	85	36.47
Unknown[20]	Triton	Polyclonal	32	37.50
A0485[33,35]	Dako	Polyclonal	310	47.42
NCL-CB11[21,44]	Novocastra	Monoclonal	541	49.91
300G-9[37]	Digiesi et al	Monoclonal	22	59.09
CB11[32,45]	Biogenex	Monoclonal	300	63.33
3B5[45]	Oncogene science	Monoclonal	176	82.39
NCL-CBE1[46]	Oncogene science	Monoclonal	35	82.86

in immunohistochemical HER2 studies. Incubation times of the antibodies in the various studies ranged from 60 minutes to overnight, and the antibody dilutions were between 1:20 and 1:250.[16,21,22,26,44] However, these variations do not entirely explain the differences in HER2 staining as no consistent correlation is observed between incubation time and overexpression. Both Kountourakis et al and Sun et al report an overexpression of approximately 22% (cytoplasmic and membranous); Kountourakis et al used 1:250 dilution and 60 minutes incubation, while Sun et al used 1:40 with an overnight incubation.[21,26] The two studies reported a highest overexpression of approximately 82%, incubation times of respectively 45 minutes and overnight respectively were used. In these studies, however, the comparability is hampered by the use of different antibodies.[45,46] We performed a comparison of incubation times in our own laboratory, incubating paraffin-embedded colorectal tumor tissue using A0485, an anti-HER2 polyclonal antibody (DAKO Glostrup, Denmark) after antigen retrieval. As shown in Figure 1, an incubation time longer than the 30 minutes indicated in the manufacturer's protocol for breast cancer tissues gives rise to a stronger cytoplasmic staining pattern, allowing better comparison between different colorectal tumors. Because the absolute amount of stainable HER2 in colorectal cancers seems low when compared to breast cancer, it seems advisable for immunohistochemical analysis of HER2-overexpression
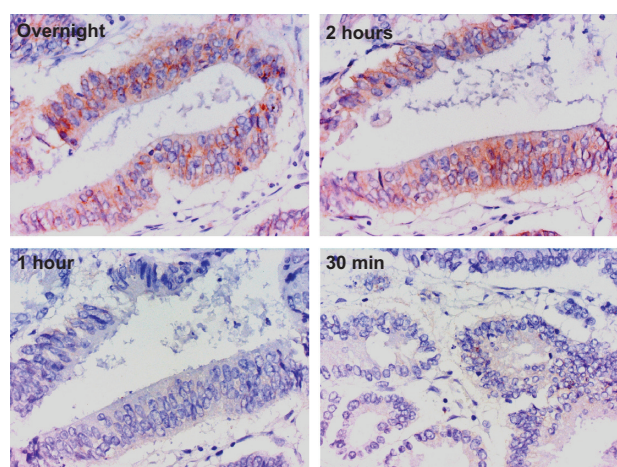

Figure 1. Paraffin-embedded colorectal cancer tissue was incubated with A0485 anti-HER2 antibody with increasing incubation times of 30 minutes, 1 hour, 2 hours and overnight.
Note: With longer incubation times, an increase in cytoplasmic staining (brown) can be observed.

to adapt the protocols for breast cancer for use in colorectal cancer.

Mirlacher et al showed a dramatic decrease in staining intensity for HER2 in tissue microarrays (TMAs) between freshly cut and stored slides.[39] We confirmed these results recently using our own TMA consisting of 500 colorectal cancer tissue samples obtained between 1991 and 2001. This TMA was produced using the same techniques as described earlier by our group for breast cancer.[40] Slides were incubated with A0485. The operation date, and therefore the length of storage time, had a great impact particularly on cytoplasmic HER2 expression. In the group of patients operated on before 1996, 8.4% showed overexpression of cytoplasmic HER2, while in the group of patients operated on in or after 1996, 42.7% expressed cytoplasmic overexpression. Tumors from patients operated on in 1998 or later showed the highest percentage of cytoplasmic staining (52%, manuscript in preparation). Therefore, it is likely that in studies having a long period of tissue collection the HER2 overexpression rate is underestimated especially in the older samples, which could partly explain the variation between studies.

These data emphasize the importance of standardized staining procedures, as relatively insignificant procedure variations might lead to completely different staining patterns and thereby different overexpression rates.

Scoring Procedure

A number of publications analyzed HER2-overexpression in colorectal tumors with genomic techniques like FISH, RT-PCR, Southern blotting, and Northern blotting. Kavanagh et al performed FISH on two 3+ cases and on nine 2+ cases. Both 3+ cases were confirmed to have gene amplification, but only one 2+ case was confirmed.[24] Nathanson et al found approximately the same results, confirming three out of three 3+ cases and one out of two 2+ cases with both FISH and PCR.[28] Ooi et al confirmed all 3+ and 2+ cases with FISH.[29] Together these results confirmed that for colorectal cancers, the observations are similar for those found in breast cancer, ie, that 3+ overexpression is conclusive for gene amplification, whereas 2+ staining is equivocal for gene amplification, and likely has other mechanisms of genetic overexpression.

When analyzing all accessible papers about HER2-overexpression on colon cancer tissue for their

scoring methods, it was observed that most publications follow the breast cancer protocol by examining only membranous overexpression,[24,25,27–31,34,36,42,43] or split their scoring in membranous and cytoplasmic overexpression.[16,18,22,23,26,41] In general, the membranous overexpression rates varied between 0% and 15%. Only Knösel et al and Park et al reported membranous overexpression of over 40%.[34,36] However, Park et al only confirmed two out of 27 3+ cases with FISH, which puts their results in a different perspective. Without these two outliers, the weighted average overexpression of membranous

HER2 was 5%. For cytoplasmic overexpression however, the rates varied between 0% and 66%, with a weighted average of 30% (Table 2).

A number of publications did not split their scores into membranous and cytoplasmic overexpression, but took them together to determine positivity.[20,21,32,33,35,37,38,45,46] The observed overexpression rates varied between 22% and 83%, with a weighted average of 50% (Table 2). Unfortunately, no conclusions can be drawn about this percentage regarding the fraction of membranous or cytoplasmic overexpression. Based on the publications that used split results, it is

Table 2. An overview of all papers reporting about HER2 overexpression in colorectal cancer. The papers are organized based on the scoring categories: split into membranous overexpression and cytoplasmic overexpression (A), or papers without distinguishing between the overexpressing regions (B). The weighted average was calculated with and without outliers. When one paper used more than one antibody, all antibodies were examined separately for the weighted average.

(A) Membraneous and cytoplasmic HER2 overexpression evaluated separately

Authors	Year	Antibody	Patients (n)	% membrane	% cytoplasm
Kay et al[16]	1994	NCL-CB11	164	0	34
Arnaout et al[41]	1992	4D5, 21N, HER14	70	0	7–34
Kim et al[25]	2004	Herceptest	185	1	
Osako et al[18]	1998	Nichirei	146	2	66
Nathanson et al[28]	2003	Herceptest	169	3	
Ooi et al[29]	2004	Nichirei	244	3	
Schuell et al[30]	2006	Herceptest	77	4	
McCann et al[42]	1990	21N	23	4	
Half et al[23]	2004	e2-4001 and 3B5	96	5	64
Ochs et al[31]	2004	Herceptest	109	6	
Kountourakis et al[26]	2006	NCL-CB11	106	6	17
Gill et al[22]	2011	NCL-CB11	40	8	58
Kavanagh et al[24]	2009	4B5	106	10	
Dursun et al[43]	2001	CB11	86	14	
Li et al[27]	2011	Herceptest	317	15	
Park et al[36]	2007	Z4881	137	47*	
Knosel et al[34]	2002	A0485	74	51*	
Total % positive membrane				9.22	
Total % positive membrane without outliers (*)				5.38	
Total % positive cytoplasm					30.58

(B) Membrane and cytoplasm evaluated together

Authors	Year	Antibody	Patients (n)	% positive staining
Sun et al[21]	1995	NCL-CB11	293	23
Demirbas et al[32]	2005	CB11	124	36
Uner et al[38]	2005	e2-4001	85	36
Kluftinger et al[20]	1992	Triton	32	38
Kruszewski et al[35]	2010	A0485	202	47
Jesus et al[33]	2005	A0485	108	48
Porcelli et al[37]	2001	300G-9	22	59
McKay et al[44]	2002	NCL-CB11	248	82
Kapitanovic et al[45]	1997	3B5 and CB11	176	82
Maurer et al[46]	1998	NCL-CBE1	35	83
Total % positive staining				55.76

expected that the majority of overexpression in these publications is located in the cytoplasm, and only a minor fraction is overexpressed in the membrane.

Overall, it can be concluded that about 5% of colorectal tumors have membranous overexpression of HER2, while the cytoplasmic overexpression varies strongly with an average around 30%. However, it is likely that this value is an underestimation due to the loss of HER2 antigen in older tissue samples as described earlier.

Cytoplasmic HER2

The FDA-approved scoring system for breast cancer is based entirely on membranous HER-2 overexpression. Cytoplasmic overexpression in breast cancer occurs, but there are strict guidelines to ignore this.[48,49] These guidelines are very specific as trastuzumab is only capable of binding the extracellular domain of membranous HER2. In other words, cytoplasmic HER2 would not have any consequence for clinical decisions about therapy. For years cytoplasmic overexpression of HER2 has been regarded as not relevant for breast cancer. Intracellular HER2 cannot be targeted by trastuzumab, it does not correlate with HER2 mRNA levels,[51] it does not correlate to any clinical outcome,[52] and it may even be a different protein than membranous HER2.

In contrast to breast cancer, there is evidence that in colorectal cancer cytoplasmic HER2 could in fact be associated with survival prognosis. Of 15 publications that analyzed clinical prognostic parameters, six showed a worse patient survival with cytoplasmic tumor overexpression of HER2.[16,18,22,32,34,45] Seven studies showed no correlation with any clinical parameter.[20,21,26,33,35,44,46] Two other papers described a positive correlation between cytoplasmic overexpression and well-differentiated tumors.[23,38] Both publications hypothesize that enhanced cytoplasmic HER2 is an early marker in colorectal cancer, but decreases with progressing tumor stage and tumor dedifferentiation. This seems contradictory to the findings that patients with a HER2-cytoplasmic overexpressing tumor have a decreased survival rate as patients with progressed tumor (higher stage, dedifferentiation) would have less HER2 expression, but poorer prognosis. Moreover, one publication showed a worse differentiation and more lymph node metastases in cytoplasmic HER2-overexpressing tumors.[32]

When combining the distinction in scoring methods within those studies that report a clinical correlation, a remarkable pattern becomes clear. Of all seven studies that report a lack of clinical correlation, six of them do not split their scoring into membranous and cytoplasmic staining.[20,21,33,35,44,46] In contrast, out of the six studies that report a clinical correlation, four of them do split their results in membranous and cytoplasmic staining.[16,18,22,34] Although this has never been confirmed in a large multi-center trial, these studies strongly suggest a clinical relevance of cytoplasmic HER2 overexpression in colorectal cancer.

Assuming that cytoplasmic HER2 has a prognostic role in colorectal cancer, it might also be involved in tumor pathogenesis like membranous HER2 in breast cancer. A plausible explanation would be that cytoplasmic HER2 is forming homodimers, leading to an intracellular activation of the tyrosine kinase domain. Administration of trastuzumab will therefore not have any effect, as this antibody targets the extracellular domain only. Lapatinib, an intracellular tyrosine kinase inhibitor, has recently been approved for treatment of HER2-positive breast cancer patients.[50] If cytoplasmic HER2 is indeed actively involved in colorectal carcinogenesis the administration of lapatinib, or other intracellular HER2-targeting compounds, could be a breakthrough in the treatment of especially this type of cancer.

Biology of cytoplasmic HER2

In breast cancer, it was suggested that cytoplasmic HER2 is a truncated or different protein, based on the fact that a 155 kD peptide was found, in contrast to the 185 kD HER2 found on the membrane. However, for colorectal cancer there have been ambiguous observations. Osako et al performed a western blot analysis on one membranous overexpressing specimen, five membranous cytoplasmic specimens, and one negative sample. They observed 185 kD and 155 kD peptides in the membranous overexpressing specimen, but only 155 kD peptides in the cytoplasmic overexpressing specimens.[18] On the other hand, Kapitanovic et al performed a study in which they were able to split the cell lysate into a cytoplasmic and membranous fraction, and performed a western blot analysis with a different anti-HER2 antibody than Osako et al. They observed a 185 kD peptide in both fractions, and reported no 155 kD peptide. A western

blot using a P-Tyr antibody, detecting phosphorylated (activated) tyrosine kinase domains showed a 185 kD peptide in the intracellular fraction, indicating an intracellular activated HER2-receptor.[45] This would suggest that the activated HER2 was internalized upon activation, or that HER2 was activated intracellularly eg, via homodimerization. Half et al analyzed the localization of cytoplasmic HER2 in colorectal cancer by electron microscopy after labeling with an anti-HER2 antibody and a secondary colloidal gold-labeled antibody. HER2 was located in the rough endoplasmatic reticulum (rER), consistent with newly synthesized peptides. It was also detected in cytosolic vacuoles, at the plasma membrane, and at desmosomes in the intercellular junctions. Particularly because of the localization at the rER, it is likely that this HER2 is derived from original peptide synthesis, and thus would be of genomic origin. Most likely, however, HER2 internalization and synthesis are occurring at the same time. Until these experiments are repeated using an antibody which has been validated to be solely specific for HER2, the origin of cytoplasmic HER2 will not be clarified.

Based on the hypothesis that cytoplasmic HER2 in colorectal cancer is actually newly synthesized rather than internalized, three different studies analyzed genomic amplification to determine the genomic background of HER2-overexpression. Half et al discovered membranous overexpression in only five out of 96 colorectal tumors (5%), while they determined cytoplasmic overexpression in 61 tumors (63%). Four out of five membranous HER2-overexpressing tumors showed amplification with FISH, while no cytoplasmic HER2-overexpressing tumors were confirmed with this technique. Moreover, RT-PCR was performed and showed a 12-fold higher mRNA expression in membranous overexpressing tumors compared to cytoplasmic overexpressing tumors.[23] Pavlakis et al performed FISH-analysis on all cases described by Kountourakis et al. All 3+ cases of membranous overexpression were confirmed as being amplified, while only one of three 2+ cases was confirmed. No single cytoplasmic overexpressing tumor was confirmed.[26,54] These results clearly indicate that membranous overexpression is associated with gene amplification, while cytoplasmic overexpression is due to other mechanisms. Maurer et al performed a northern blot and a Southern blot on the 29 cases overexpressing HER2, without a distinction between membranous and cytoplasmic overexpression. Presumably this concerns cytoplasmic HER2 based on the high percentage of positive tumors (82%). They observed a 1.5 fold increase in mRNA via northern blot in tumors compared to healthy tissue, but the Southern blot did not show any gene amplification.[46] Again, this indicates that cytoplasmic HER2 overexpression is based on mechanisms other than gene amplification.

For other types of cancer, it has been shown that overexpression of HER2 can also occur via mechanisms other than gene amplification, eg, via increased levels of promoter-binding proteins as was observed by Kameda et al in gastric cancer. He observed elevated levels of binding proteins to the TATA-box located in the promoter-region of the HER2-gene, which led to overexpression of HER2.[55] Theoretically, mutations in downstream targets of HER2, for example KRAS, might also influence the expression of HER2 via affected feedback processes. These mechanisms could play a role in the observed cytoplasmic HER2 overexpression without any gene amplification. However, it is still unknown why HER2 does not migrate to the cell membrane, as it should being a transmembrane receptor. Whether it is a simple matter of quantities, and whether this transcriptional upregulation result in not enough HER2 to migrate to the cell membrane is unknown. It is controversial as the western blots from Kapitanovic et al and Osako et al exhibit a much denser signal when analyzing the cytoplasmic overexpressing cells, when compared to the membranous overexpressing cells.[18,45] However, the 12-fold higher mRNA expression in membranous HER2 overexpressing tumors compared to cytoplasmic overexpression as discovered by Half et al would support this theory. Perhaps there is an unknown HER2-transporter protein which is also regulated by the promoter-binding protein. Or perhaps the protein is in some way truncated when being overexpressed at a transcriptional level. Other possibilities include there being an actual 155 kD cleavage product incapable of migrating to the membrane or an immune-evasive strategy by the tumor. One theory is based on the influence of the unfolded protein response (UPR). Upon cellular stress, this response initially stimulates multiple chaperone proteins in the endoplasmic reticulum

in order to increase the glycosylation and folding capacity of the endoplasmic reticulum, and induces apoptosis when this initial response is insufficient. UPR activation in breast cancer cells has been demonstrated.[56] In normal, non-malignant cells there is a baseline expression of HER2, which leads to a HER2 on a cell membrane that cannot be detected by regular immunohistochemistry (Fig. 2A). A possible theory is that in the case of overexpression of the HER2-gene, there is a surplus of HER2 mRNA which, after being translated into protein, cannot be handled properly in the ER and thus leads to aberrant glycosylation or misfolding of the immature protein (Fig. 2C). In this case, either the Golgi apparatus is not capable of maturing the protein or the protein cannot be transported to the Golgi apparatus, both of which lead to accumulation of aberrant HER2 within the cytoplasm. This accumulation would trigger the UPR. The presence of an activated UPR will lead to an increase in properly glycosylated and folded protein, and the overexpression of HER2 by the cell at the membrane. However, for an unknown reason, gene amplification of HER2 in breast cancer leads to a more efficient UPR than HER2-overexpression in colorectal cancer (Fig. 2B). This difference in UPR could be influenced by other processes which affect cellular stress such as hypoxia, nutrient availability, treatment, or other genetic instabilities. This may lead to the situation that UPR in breast cancer is efficient enough to process the surplus of immature
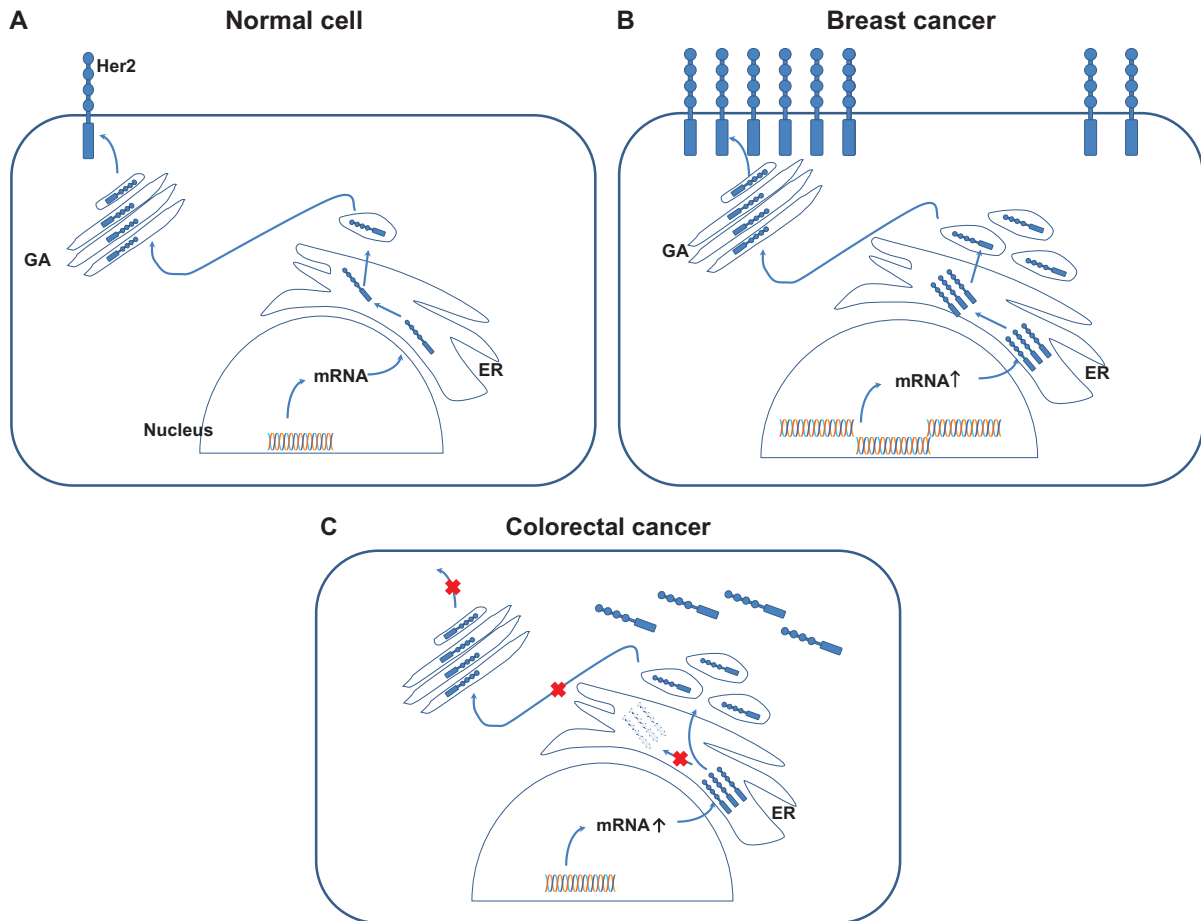

Figure 2. A schematic representation of the difference in HER2 expression between multiple cell types. In a normal cell (**A**), a single copy of the HER2-gene is transcribed to low levels of mRNA, which is then translated to HER2 which enters the endoplasmatic reticulum (ER). In the ER, HER2 is processed (eg, glycosylated), and transported to the Golgi apparatus (GA). There it undergoes final processing before being transported to the membrane. In breast cancer (**B**), this process is basically identical except that gene amplification leads to higher mRNA and HER2 levels. The processing capacity of the ER is improved, most likely due to the unfolded protein response (UPR). In colorectal cancer however (**C**), there is no gene amplification, but gene expression is upregulated during transcription.
Notes: The ER cannot keep up with the high amount of protein, therefore immature HER2 cannot to be completely processed in the ER, and cannot be transported to the GA and the cell membrane; instead incompletely folded HER2 might be extracted from the ER via the ER associated degradation pathway, deglycosylated into a 155–160 kDa protein prior to it accumulation and ultimately proteasomal degradation in the cytoplasm.[58]

HER2, while in colorectal cancer the UPR fails to do so which leads to accumulation of misfolded and aberrantly glycosylated HER2. This unglycosylated form of HER2 might be identical to the p155 variant mentioned before.

Conclusions

It is clear that most of the debate around HER2-overexpression in colorectal cancer is due to differences in staining/scoring techniques. When splitting the results in membranous and cytoplasmic overexpression, a clear pattern appears with approximately 5% of all patients having membranous overexpression, and about 30% of all patients having cytoplasmic overexpression. There is a strong correlation between genomic amplification and membranous overexpression, whereas no genomic amplification is observed in cases with cytoplasmic HER2 expression.[23,26,54]

With around 5% of all colorectal cancers overexpressing HER2 on their membrane, this would create a new treatment strategy with trastuzumab for approximately 60,000 patients worldwide per year. Unfortunately, a clinical trial was ended due to a lack of patient accrual.[57] A large, multicenter trial would be needed to include enough patients for sufficient analysis.

In contrast to membranous HER2 overexpression, a significant proportion of colorectal tumors (30%–50%) show cytoplasmic HER2 overexpression in most studies. The identity of cytoplasmic HER2 remains unclear, although there is some indirect evidence that it is derived from the upregulation of promoter-binding proteins leading to an increase in HER2 production. Additionally, the prognostic value of cytoplasmic HER2 is still unclear. An extensive study using standardized techniques on a large number of tumors is needed to elucidate this matter. If cytoplasmic HER2 has a pathophysiological role in colorectal cancer, intracellular HER2-targeting compounds, for example lapatinib, might be a new treatment option for the 30% of patients having cytoplasmic HER2 overexpression. This would impact about 360,000 patients per year; a clear breakthrough in the treatment of colorectal cancer.

Author Contributions

Conceived and designed the experiments: EB, CS. Analysed the data: EB. Wrote the first draft of the manuscript: EB. Contributed to the writing of the manuscript: EB, PK, CS. Agree with manuscript results and conclusions: EB, PK, JvL, CS. Jointly developed the structure and arguments for the paper: EB, PK, JvL, CS. Made critical revisions and approved final version: EB, PK, JvL, CS. All authors reviewed and approved of the final manuscript.

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Competing Interests

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