ITEM OF CORRESPONDENCE

Title

HER2 protein overexpression in non-amplified ductal carcinoma in situ: quality issue or transcription mechanisms gone awry?

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This article has been accepted for publication and undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process, which may lead to differences between this version and the Version of Record. Please cite this article as doi: 10.1111/his.13799

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Keywords

Ductal carcinoma in situ, HER2, amplification, protein overexpression, discordance

Short running title

Discrepant HER2 status in DCIS

Conflict of interest statement

The authors declare no conflicts of interest.

Letter to the editor

Sir, we read with interest the recent report by Hui et al. entitled 'Discordant HER2 immunohistochemical expression and gene amplification in ductal carcinoma *in situ* – evaluating HER2 in synchronous in situ and invasive carcinoma'.¹ This study compares HER2 immunohistochemistry (IHC) and chromogenic *in situ* hybridization (CISH) between admixed synchronous in situ and invasive carcinomas exhibiting an equivocal 2+ HER2 IHC score in the invasive component. The authors are to be commended for their thorough analysis, which revealed that 31 out of 37 DCIS (84%) with a genuinely positive 3+ HER2 IHC score did not present *HER2* amplification. However, we disagree with their final conclusion: the authors state that IHC-based HER2-positive DCIS might lack an underlying molecular basis (i.e. absence of *HER2* gene amplification) and consequently, that immunohistochemistry should be used with caution for determining HER2 status in DCIS. We hypothesize that HER2 protein overexpression in DCIS is likely to yield a higher predictive value than *HER2* gene amplification, since HER2-targeted therapies target the HER2 protein instead of the *HER2* gene. Nevertheless it should be noted that there is presently no evidence for the use of HER2-targeted therapies in DCIS without synchronous HER2-positive invasive carcinoma, and therefore evidence to support this hypothesis is lacking.

At present, HER2 protein overexpression can be of diagnostic relevance in DCIS, such as in the differential diagnosis of associated Paget's disease. HER2 immunohistochemistry can be used to discern Toker cell hyperplasia, which is HER2-negative, from mammary Paget's disease, which is almost always HER2-positive. In this situation, discrepancies between immunohistochemistry and ISH will not be encountered, as the differential diagnostic work-up is unlikely to extend beyond immunohistochemistry.

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At first sight, the observations of Hui et al. may seem paradoxical and might raise questions on technical quality issues and misinterpretation of IHC. Yet we can corroborate their findings, as we previously reported similar results on HER2 IHC and fluorescence *in situ* hybridization (FISH) in a cohort of pure DCIS and DCIS admixed with invasive breast cancer.² In our series, seven of forty-one pure DCIS (17%) and seven of nineteen admixed DCIS (37%) with a genuinely positive 3+ IHC score presented without *HER2* copy number-based amplification.² Since all these IHC and FISH tests were performed in an ISO15189 accredited laboratory and since independent researchers like Hui et al. observed a similar phenomenon,¹ this paradoxical discrepancy between IHC and ISH testing in DCIS is unlikely to be attributed to mere qualitative technical issues. Others have observed similar discrepant results. When HER2 assessment was still in its infancy, Slamon et al. reported that about ten percent of invasive breast cancers showed clear overexpression at the mRNA and protein levels while these tumours were judged as single copy by DNA analysis.³ At that time, it was suggested that these tumours may possess alterations in control mechanisms for gene expression instead of an increased gene copy number.³ Despite the rare discrepancies between protein overexpression and gene amplification status in invasive carcinoma, this phenomenon is more commonly observed in DCIS, regardless the presence of synchronous invasive carcinoma. Although the ASCO/CAP guidelines on HER2 testing recommend to aspire >95% concordance rates for IHC and ISH results, this recommendation may be too stringent for DCIS. The study of Hui et al. as well as our own observations show that discordant ISH and IHC results in DCIS occur quite frequently.^{1,2}

Accumulating *in vitro* evidence shows that several transcription factors might be involved in protein overexpression in absence of *HER2* amplification. Constitutively activated STAT3 has been described as a potential cause of increased HER2 protein expression, since a *cis*-acting element in the *HER2* promoter is regulated by STAT3.⁴ Powe et al. reported that nuclear AP-2 α/β expression correlates strongly with nuclear YY-1 positivity in a large series of breast cancers, and both AP-2 α/β and YY-1 expression correlate significantly with HER2 protein expression.⁵ Although discordant *HER2* gene amplification and protein expression was a rare event in their TMA-based study, four of these six discordant cases displayed nuclear AP-2 expression.⁵ *In vitro* experiments demonstrated that YY1 and Ku proteins cooperate with AP-2 to enhance *HER2* transcription.⁶ Since AP-2 expression is not able to account for all discrepancies, other transcription factors might be involved too. More research is required to explore this phenomenon. Series of discrepant cases such as the one reported by Hui et al. could therefore offer excellent opportunities to further investigate alternatively underlying molecular mechanisms.

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In conclusion, *HER2* gene amplification has long been considered a prerequisite for HER2 protein overexpression, but perhaps the time has come to adjust this dogma. It is likely that HER2 overexpression in DCIS is the result of both gene amplification and enhanced gene transcription, with a minority of HER2-positive DCIS lacking gene amplification but displaying protein overexpression due to transcription mechanisms gone awry. We should therefore not contest the value of HER2 IHC in DCIS. Instead, we should question the stringent 95% concordance rule of the ASCO/CAP recommendations for invasive breast cancer, as not all discordant DCIS seem to be due to technical errors or misinterpretation of staining. The 95% minimum concordance requirement strictly refers to invasive breast cancer. It seems unlikely that it can be extended to DCIS. Evidence is limited for the time being, but it suggests underlying biological mechanisms for these discrepancies nonetheless.

Acknowledgements

The content of this letter has been discussed by all authors. All authors have reviewed and edited this manuscript.

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