gastric foveolar type, through loss of cell-to-cell adhesion by the destruction of cell-adhesion molecules, represented by E-cadherin.

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CD95 ligand expression as a criterion of malignant transformation in breast cancer

With great interest we read the article by Müllauer *et al*. on CD95 and CD95 ligand (CD95L) expression in breast cancer and normal mammary epithelia [1]. The role of CD95 and its ligand in breast cancer progression was the subject of extensive study and is now well established [2–5]. One of these studies was also published last year in the *Journal of Pathology* [2]. Nevertheless, the authors do not compare their data with the results of any of those previous studies. The report by Müllauer *et al.* largely overlaps with previously published findings. In contrast with previous work, however, the authors claim that normal mammary epithelia are also a source of CD95L expression. This claim requires further consideration as it contradicts the emerging concept that CD95L expression in breast cancer is specific for and restricted to the (pre-)malignant cells [2–5] and is thus a criterion of malignant transformation of mammary epithelial cells.

Given that infiltrating T cells usually express CD95L, the purity of the analysed tissue is critical and appropriate controls are mandatory. For this reason, we analysed only tissue samples lacking obvious T-cell infiltration, as assessed by staining for CD3 expression. The absence of significant T-cell infiltrates was further controlled by the determination of CD3δ mRNA copy numbers in all samples by quantitative competitive RT-PCR (co-amplifying an internal DNA standard). Eliminating T cells as a possible source of 'contamination', we found CD95L expression exclusively in breast cancer cells. The CD95L mRNA copy numbers varied as a function of the degree of dedifferentiation of the tumour cells. The problem of infiltrating T cells was also encountered and thoroughly controlled by two other groups [3,4].

Applying a 'semiquantitative' PCR approach without an internal DNA standard in 38 amplification cycles (i.e. amplification to saturation), Müllauer *et al.* do not approriately address this issue, as under these amplification conditions a few contaminating T cells would also give rise to a PCR product. Indeed, mRNA and immunohistochemical data were inconsistent, as the authors themselves point out.

Müllauer *et al.* also studied CD95L expression in three 'normal breast epithelial cell lines'. Given that these cell lines were grown under long-term cell culture conditions and immortalization was induced by prolonged treatment with benzo[a]pyrene, it appears elusive that they might still reflect the gene expression pattern of the 'normal' counterpart of breast cancer. In fact, for some of these 'normal' mammary epithelial cell lines, allelic loss of chromosome 9p and mutations of the p53 gene have been demonstrated [6,7]. Finally, any type of immortalization may have significant impact on the 'normal' expression pattern of the CD95 gene and its ligand: for instance, infection of human B cells with the Epstein–Barr virus (which mostly occurs in the absence of any pathology) induces B-cell immortalization and *de novo* expression of CD95L in the immortalized B cells [8]. Unquestionably, these $CD95L^+$ B cells, although not malignant, no longer reflect the 'normal' phenotype of the B lineage.

The work by Müllauer *et al.* thus seems to confirm that CD95L can be expressed by breast cancer cells. For their claim, however, that normal mammary

epithelial cells also express CD95L, there is to date no evidence.

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Author's reply

We appreciate the opportunity to respond to the letter by Müschen and Beckmann concerning the expression of Fas ligand (FasL) in normal (non-malignant) and malignant human breast epithelial cells. The authors criticize the fact that we did not discuss their publication [1] or the work of two other laboratories [2,3] on FasL expression in breast cancer cells. We did not compare our data [4] with the results of these studies because they were published either after submission [2,3] or after the proofreading [1] of our revised manuscript.

Müschen and Beckmann claim that FasL expression in breast cancer is 'specific for and restricted to the (pre-)malignant cells and is thus a criterion of malignant transformation in mammary epithelial cells'. In contrast to that view, our data indicate that FasL is also expressed by normal (non-malignant) breast epithelial cells (obtained from reduction mammoplasties) and in benign breast disease (fibrocystic changes, fibroadenoma), although FasL expression was mostly weak and restricted to a minority of epithelial and myoepithelial cells [4].

Müschen and Beckmann point out that we did not exclude infiltration of breast tissues by FasL-positive T-cells in the RT-PCR reactions. We agree that by using RNA derived from homogenized breast tissue, contaminating T-cells may contribute to the generation of a positive signal.

Müschen and Beckmann furthermore indicate that the normal (non-malignant) breast epithelial cell lines (184B5, MCF-10A, MCF-12A) used in our study were benzo[a]pyrene-treated and some of them exhibit allelic loss of chromosome 9p [5] and mutations of the p53 gene [6]. In fact, only one cell line (184B5) is benzo[a]pyrene-treated; two (MCF-10A, MCF-12A) are spontaneously immortalized. All three cell lines are derived from benign breast tissues. Allelic loss of 9p was described for 184B5 and MCF-12A [5] and elevated levels of p53 were detected in 184B5, but without the presence of a mutation in the analysed exons 4–9 [6].

We detected FasL mRNA and protein by RT-PCR and western blotting in these three cell lines [4]. A course, immortalized cell lines may not fully reflect the phenotype of normal (non-malignant) cells *in vivo*, but the strongest indication for FasL expression in normal (non-malignant) breast epithelial cells is derived from our immunohistochemical studies [4]. Furthermore, FasL protein expression in normal (non-malignant) breast epithelial cells was also observed by Gutierrez *et al*. [2] in the vicinity of breast carcinomas and in hyperplastic breast tissue, using a different antibody.

In summary, concluding from our data and the results of Gutierrez *et al.* [2], FasL expression is not restricted to breast cancer cells but is also observed in normal (non-malignant) breast epithelial cells, although usually at much lower levels.

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