exhibited more than five signals per nuclei or large gene signal clusters [8,34,36].

Out of the 112 cases of metaplastic breast carcinomas in our series, 25 had sufficient material in the blocks and were successfully analyzed by both immunohistochemistry and CISH for EGFR and HER2.

Correlation between EGFR overexpression and amplification and clinicopathological parameters and survival

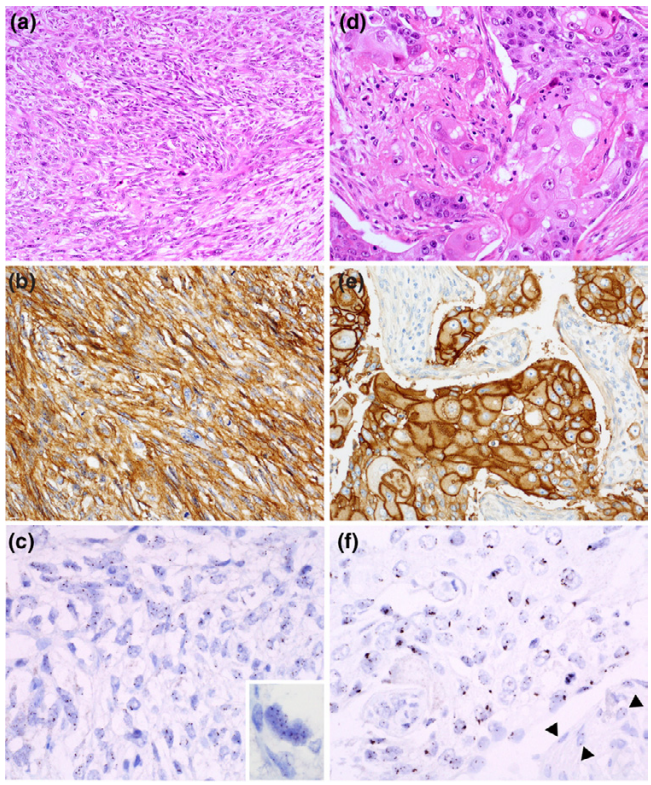
Follow-up information was available for 23 out of 25 patients, with follow-up periods ranging from 5.5 to 124.3 months (median 34.6 months, mean 51.9 months). The Statview software package was used for all calculations. Correlations between categorical variables were performed using the χ^2 test and Fisher's exact test. Correlations between continuous and categorical variables were performed with analysis of variance. DFS and OS were expressed as the number of months from diagnosis to the occurrence of an event (local recurrence/metastasis and disease-related death, respectively). Cumulative survival probabilities were calculated using the Kaplan–Meier method. Differences between survival rates were tested using the log-rank test. All tests were two-tailed, with a confidence interval of 95%.

Results

Table 1 summarizes the results of the histological, immunohistochemical and CISH analyses. Briefly, 19 out of 25 (76%) cases exhibited EGFR positivity of grade 3+. No samples showed EGFR grade 2+ positivity, whereas four cases were EGFR 1+. Out of the 19 cases with EGFR grade 3+ expression, seven (37%) exhibited *EGFR* gene amplification (Fig. 1). Three out of nine carcinomas with squamous metaplasia and four out of 10 spindle cell carcinomas had *EGFR* amplification, whereas no matrix producing breast carcinomas showed any amplification. Interestingly, similar numbers of *EGFR* signals were observed in the epithelial and in the metaplastic elements. One case exhibited HER2 grade 2+ positivity with Herceptest®, but *HER2* gene amplification was not observed (Fig. 2).

No association between EGFR overexpression or amplification and clinicopathological features was observed (Table 2). EGFR overexpression showed no association with DFS or OS. Patients with tumours harbouring EGFR amplification had a trend toward shorter DFS and OS (Fig. 3).

Figure 1



EGFR overexpression and gene amplification in MBCs. Photomicrographs of **(a)** a spindle cell metaplastic breast carcinoma (haematoxylin and eosin) showing **(b)** grade 3+ immunohistochemical positivity for EGFR and **(c)** *EGFR* gene amplification (>5 signals per nucleus [CISH]). Inset in panel c: note the bizarre neoplastic cell with more than 10 copies of *EGFR*. **(d)** Breast carcinoma with squamous metaplasia (haematoxylin and eosin) with **(e)** EGFR grade 3+ immunohistochemical positivity. **(f)** CISH demonstrating *EGFR* amplification (clusters of signals in the nuclei of neoplastic cells). Note the presence of one or two signals in the nuclei of stromal cells (arrowheads). CISH, chromogenic *in situ* hybridization; EGFR, epidermal growth factor receptor; MBC, metaplastic breast carcinoma.

Discussion

In recent studies, we and others demonstrated that MBCs frequently overexpress EGFR and lack HER2 overexpression. In a previous study [26] we demonstrated that up to 83% of all MBCs show EGFR overexpression. Leibl and Moinfar [12] described positivity for EGFR in 70% of MBCs, but those authors also considered cases with grade 1+ expression to be positive. When only grade 2+ and 3+ expression was considered to represent positivity, 60% (12/20) were positive. In the present study we demonstrated that 76% (19/25) of MBCs overexpressed EGFR. The differences between our findings and those of Leibl and Moinfar [12] may be related to the different antibody clones used and different antigen retrieval methods.

The mechanism underlying EGFR overexpression has not been investigated in MBCs. In the present study we demonstrated that *EGFR* is amplified in 28% (7/25) of MBCs and in 37% (7/19) of MBCs with EGFR overexpression. Although only six cases with heterologous elements (four matrix producing carcinomas and two carcinomas with heterologous elements) were analyxed, no amplification was found in these two subtypes of MBC. Identification of areas of squamous differentiation in spindle cell carcinomas and the presence of spindle