the treatment well. After treatment, the surface of the tumour became oedematous and ulcerated over the next 24 h. Biopsies were done at 48 h to see if photodynamic necrosis had occurred. Specimens were examined by an oral pathologist with experience of photodynamically-induced injury. Characteristic coagulative necrosis with evidence of microvascular injury was seen in the 3 patients. Treatment was palliative and complete tumour ablation was not an aim in these advanced cancers. Treatment with ALA did not cause abnormalities in electrolyte balance nor haematological indices. 3 of the 4 patients showed a rise of serum aspartate aminotransferase concentrations, which returned to normal within 3 days. 2 had a small temporary rise in total bilirubin.

This study shows that oral squamous cell carcinomas can synthesise and accumulate photosensitising levels of PPIX after oral ALA. Only tumour samples were removed from the mouth to measure fluorescence, and normal tissue sensitisation was not examined, although some necrosis of normal mucosa next to the tumour and exposed to light was seen. The main advantage of using ALA-induced PPIX for photosensitisation is the rapid clearance of the sensitiser within 24 h, which would make it possible to repeat treatments at short intervals until ablation is complete. Giving ALA by mouth is more acceptable to the patient than by intravenous injection. The use of systemic ALA to induce PPIX photosensitisation is a novel and promising method of tumour photodynamic therapy which may prove suitable as a primary treatment for early cancers. Further studies are justified to find out the best drug-light combinations.

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Secretion of epidermal growth factor by macrophages associated with breast carcinoma

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By means of a cytokine release assay we have shown that in cell populations derived from primary breast carcinoma, epidermal growth factor (EGF) is secreted by cells with the characteristic morphological and immunophenotypic profile of activated macrophages (positive for CD68, CD16, CD25). EGF secretion was observed in 11 (31%) of 35 primary tumours. Secretion of EGF by normal or malignant epithelial cells was not observed. We found no association between EGF secretion by the primary tumour and recognised clinical indices of prognosis.

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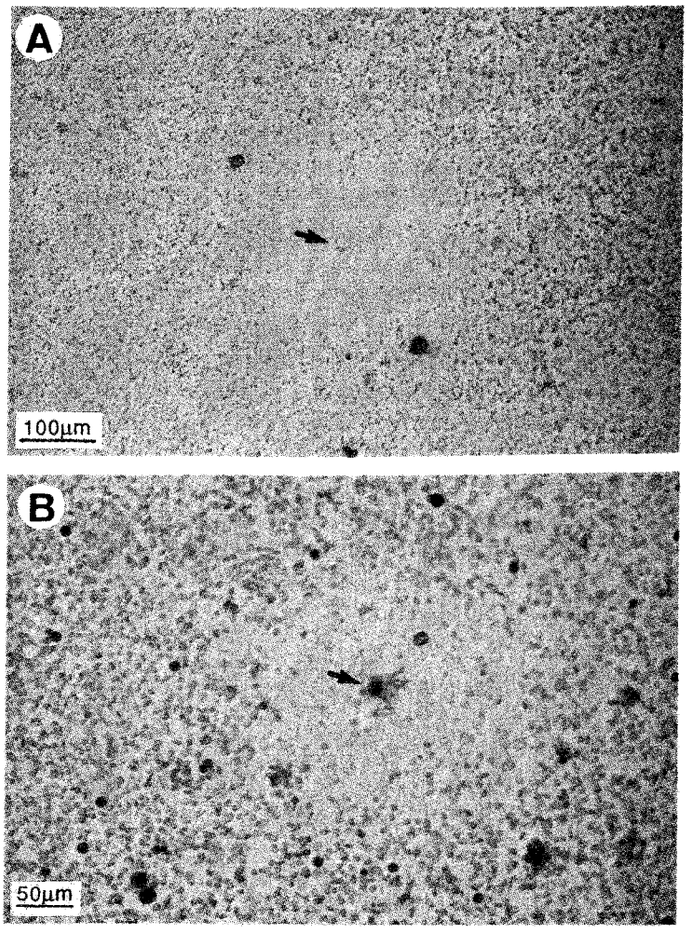
It is well known that cell proliferation can occur by way of autocrine and paracrine interactions of growth factors with their receptors. What is less clear is the contribution of these mechanisms to the development and progression of tumours.[1] In solid tumours and their metastases, both malignant cells and normal cells within the tumour stroma are potential sources of growth factors. Tumour-associated macrophages (TAM) constitute a large proportion of the



Immunophenotyping of EGF-secreting cells derived from breast carcinoma biopsy samples

A: EGF-secreting cell (arrow) negative for immunocytochemical markers of normal/malignant epithelial cells (Cam 5 2/HMFG-2). Positively stained cells (red) visible outside area of erythrocyte lysis.
B: EGF-secreting cell (arrow) also negative for epithelial markers Cam 5 2/HMFG-2 (brown) but positive for pan-macrophage marker CD68 (red).

Feature	EGF-secreting (n=11)	Non-EGF-secreting (n=24)
Mean age in yr (range)	51 6 (27–68)	60 2 (38–86)
Mean (SE)		
Oestrogen receptor concentration (fmol/mg)	32 2 (14 0)	84 2 (32 9)
EGF receptor concentration (fmol/mg)	28 2 (4 4)	39 6 (10 5)
Tumour size (cm)	3 9 (0 6)	4 7 (0 6)
No (%) patients with nodes involved	7 (64%)	14 (58%)

Table: **Clinical features of tumours positive or negative for EGF secretion**

cells infiltrating solid tumours (up to 80% in breast carcinoma). The function and possible role of these cells in disease prognosis is controversial.[2] If growth factors contributing to tumour progression are released by TAM, therapeutic approaches against these cells may be possible.

We have investigated the secretion of epidermal growth factor (EGF) by cells derived from primary breast carcinoma. To identify the cells secreting EGF we used the reverse haemolytic plaque assay (RHPA), which detects the release of soluble factors by individual immunophenotyped cells.[3]

Tumour biopsy samples were obtained from 35 women with breast carcinoma (31 ductal, 1 lobular, 1 mucinous, and 2 mixed lobular/ductal). After enzymic disaggregation of tumours, cell populations comprising both tumour-infiltrating and malignant cells were applied to the RHPA as described previously.[3] EGF secretion was detected as an area of erythrocyte lysis around the central secreting cell (figure).

11 (31%) of the 35 primary breast carcinomas showed EGF release. The secreting cells did not have morphological features typical of tumour cells; rather, they resembled non-neoplastic mononuclear cells. This observation was confirmed by immunophenotypic analysis of secretory cells. None of the EGF-secreting cells was of normal or malignant epithelial origin as determined by a cocktail of antibodies against cytokeratins and milk fat globule membrane (figure, A). Instead, they were positive for leucocyte common antigen (CD45), which indicates haemopoietic origin. The phenotype of secretory cells from 3 tumours was further defined by use of standard markers of haemopoietic subsets (figure, B). In all 3 cases the immunophenotypic profile obtained was that characteristic of activated macrophages (positive for CD68, CD16, and CD25). Less than 5% of all the cells bearing this phenotype were actively secreting EGF.

We found no significant association (Mann-Whitney U test) between the presence or absence of EGF secretion in the primary tumour and known clinical features of prognosis (table).

In vitro EGF is mitogenic for a wide variety of mesenchymal and epithelial cell lines, and in vivo it has been implicated in epithelial proliferation and repair.[4] The role, if any, of EGF in the regulation of breast cancer growth remains unclear. Immunohistochemical studies have located EGF to the epithelial component of a small number of breast tumours[5] and mRNA for EGF has also been isolated from breast carcinoma biopsy samples.[6] Until now, however, release of the protein has not been described. We have shown that in some breast tumours EGF is secreted by macrophages associated with the tumour but not by normal or malignant epithelial cells. This finding is important since we have identified the source of secretion; immunohistochemical analysis cannot discriminate between synthesised protein destined for release and protein released elsewhere that is bound to cellular receptors.

That only a small proportion (<5%) of TAM were engaged in secretion suggests that these cells represent a subpopulation of macrophages with distinct functional properties and that they may have a stimulatory effect on tumour growth. Expression of the EGF receptor on the epithelial component of tumours confers a poor prognosis and is associated with hormone resistance.[7] Thus, in patients positive for EGF receptors, macrophages of this type may be especially detrimental. In this study we found no significant association between the presence or absence of EGF secretion and EGF or oestrogen receptor status. Further studies on larger numbers of tumours are needed to verify these findings.

Whalen[8] has suggested that factors released by malignant cells may recruit macrophages to the site of tumour invasion, where they misinterpret tumour-derived signals for those required in normal wound healing. In consequence, the macrophages themselves may then secrete growth factors that aid tumour growth. We have observed that tumour cells are often close to EGF-secreting cells (eg, figure, B). We are now examining the exact location of EGF-synthesising cells in relation to malignant cells by means of in-situ hybridisation. This approach could show whether TAM are triggered into EGF secretion by tumour-derived signals.

These findings warrant further investigation of the role taken by TAM and of whether a subpopulation of these cells are involved in the paracrine growth regulation of breast carcinoma. If our findings are confirmed, therapeutic strategies aimed at modulating macrophage function may be beneficial for some breast cancer patients.

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