Biphasic effect of thrombospondin-1 (TSP-1) in the regulation of angiogenesis in human breast carcinoma

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TSP-1 is a large extracellular matrix glycoprotein implicated in angiogenesis. Its specific role is not clear, as both stimulatory and inhibitory effects have been demonstrated in animal models (1, 2). Angiogenesis cannot be measured directly in human tumours, however, the density of the microvasculature in tissue sections has been commonly used as an index of this process. Although angiogenesis is important for tumour growth and dissemination, the value of microvascular density as a prognostic indicator in breast carcinoma remains controversial (3-5).

In order to assess the possible role of TSP-1 in angiogenesis, we have quantitated vascularity and TSP-1 expression in resected human breast carcinomas. Vascularity was assessed by four different methods following immunostaining of histological sections with antibodies to Von Willebrand factor (vWF). These methods were: (a) average microvascular density (a-MVD), (b) highest microvascular density (h-MVD), (c) microvascular volume (MVV) and (d) image analysis of stained area (vWF Area). a-MVD and h-MVD were assessed by counting the vessels that were contained within a grid covering an area of 0.476 mm². To measure a-MVD, vessels in 15-20 random fields across the section were counted. To measure h-MVD, the tumour sections were scanned for the area of highest vascular density; the number of vessels in 5 fields in this area were then counted and the highest value was taken. In both cases the counts were expressed as number of vessels per square millimetre. MVV was measured by point counting using an eyepiece graticule which contained 100 points; 15 random fields (1500 points) were counted across each section. TSP-1 mRNA expression was determined by (a) northern blotting (nb-TSP) using a 32P-labelled cDNA probe to human endothelial TSP-I, and (b) in situ hybridisation (ish-TSP) using a digoxigenin-labelled RNA probe. nb-TSP was quantitated by densitometric scanning and ish-TSP was quantitated morphometrically by point counting of 15 random fields.

We found good correlation between the four methods of assessing vascularity in the tumours (a-MVD v h-MVD, r = 0.92, p<0.001, n = 52; a-MVD v MVV, r = 0.84, p < 0.0001, n = 15; a-MVD v vWF Area, r = 0.87, p < 0.001, n = 27). Analysis of the results from 52 cases showed that there was no correlation between vascularity and any of the clinical prognostic factors studied, including age, nodal status, tumour size, differentiation, oestrogen receptor, progesterone receptor, C-erb-2 and p53. A correlation was found between the two methods of measuring TSP-1 mRNA expression (northern v ISH, r = 0.62, p = 0.014, n = 15). We also found a significant relationship between Ish-TSP and oestrogen receptor (r² = 0.60, p = 0.0007, n = 15). When results from 35 tumours analysed for nb-TSP were plotted against vascularity, a bell-shaped relationship was demonstrated between these two parameters (p=0.03). Seven tumours were randomly selected for further analysis. In histological sections of each tumour, 14-18 areas (each area measuring 0.7-1.4 mm²) were quantitated in terms of Ish-TSP and vascularity (a-MVD and MVV). The results indicated that individual tumours may fall into two distinct categories, with the relationship between TSP expression and vascularity exhibiting either a convex (Fig. 1A) or a concave (Fig. 1B) bell shape.

These results suggest that TSP-1 can have both stimulatory and inhibitory effects on angiogenesis, possibly dependent on the presence of other, yet unknown, factors. In view of the relationship between Ish-TSP and oestrogen receptor, the latter may be a possible candidate.

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Fig. 1 Relationship between TSP mRNA expression and vascularity in histological sections of breast tumours, 14-18 areas of each section were quantitated in terms of TSP-1 mRNA expression by ISH (TSP) and vascularity (MVV). Each point represents the mean of triplicate readings. (A) Tumour T1/94 showing convex bell-shaped relationship, (B) tumour T93/93 showing concave bell-shaped relationship.