

## ANALYSIS OF MICROVASCULATURE OF TRANSPLANTED RAT TUMORS USING SYNCHROTRON RADIATION MICROANGIOGRAPHY SYSTEM

In the 1970's, Folkman and colleagues [1] proposed a trail-blazing theory regarding tumor growth factors summarized as follows. Whereas an early-stage solid tumor can receive nutrients and oxygen through diffusion from mature vessels, in order for the tumor to grow larger than several millimeters, the induction of continuous angiogenesis is required (Fig. 1(a)). Since then, various *in vitro* and *in vivo* experimental models and observation methods have been developed to clarify the mechanism underlying tumor angiogenesis. However, there have been no techniques that enable the observation of micronsize tumor vessels by angiography, or the quantitative assessment of dynamic changes in the morphology of tumor vessels.

In the present study, N-nitrosomethylurea-induced rat mammary adenocarcinoma was transplanted to athymic nude rats, and tumor vessels in the transplanted tumor of 20-30  $\mu\text{m}$  size were analyzed using a synchrotron radiation microangiography system (BL20B2). Furthermore, the effects of treatment with either basic fibroblast growth factor (b-FGF, an angiogenesis promoter) or anti-vascular endothelial growth factor receptor neutralizing antibody (anti-VEGFR neutralizing antibody, angiogenesis inhibitor), or irradiation on angiogenesis and microcirculation were determined morphologically. The results were as follows. (i) The minimal diameter of observable vessels in athymic nude rats with a

tumor transplanted to the inferior epigastric wall was 20-30  $\mu\text{m}$  (Fig. 2) [2,3]. (ii) As to microvessel density (MVD) 1-4 weeks after transplantation in the nontreated group, a significant correlation existed between histologic MVD, which was calculated histologically on the basis of the quantity of vessels, and angiographic MVD, which was calculated by binarization computation ( $r = 0.944$ ,  $p < 0.01$ ) (Fig. 3) [4]. (iii) In the nontreated group, the scores for dilatation, serpiginous vessels and encasement of tumor vessels were high 1-2 weeks after transplantation, whereas the scores for retiform vessels and extravasation were high 3-4 weeks after transplantation (Fig. 1(b)). (iv) Among the treated groups, at 4 weeks after the treatment, the scores for retiform vessels in the b-FGF-treated group and for extravasation in the irradiated group were high. However, in the anti-VEGFR neutralizing antibody-treated group, the scores for retiform vessels and extravasation were low (Fig. 4). (v) Finally, the time-density curve for each group was classified into the waning or recruiting response pattern; whereas the former was observed 1-2 weeks after transplantation in the nontreated and b-FGF-treated groups, the latter was observed 3-4 weeks after transplantation in the nontreated, anti-VEGFR neutralizing antibody-treated and irradiated groups (Fig. 5).

Using a totally new microangiography system based on monochromatic X-rays, (synchrotron radiation is 100,000 times brighter than X-rays from a standard medical X-ray source), we were able to observe and quantitatively assess the morphology of 20-30  $\mu\text{m}$  tumor vessels, and results comparable to the conventional histological analyses were obtained. To date there have been no angiographic studies of the effects of treatment with an angiogenesis promoter or inhibitor, or irradiation on morphological changes in the tumor vessels, i.e., existing and

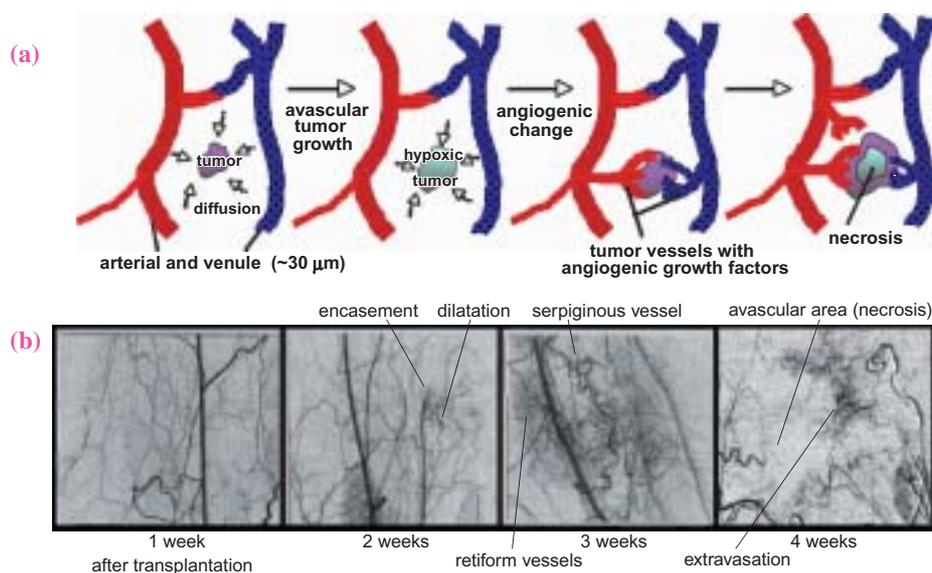
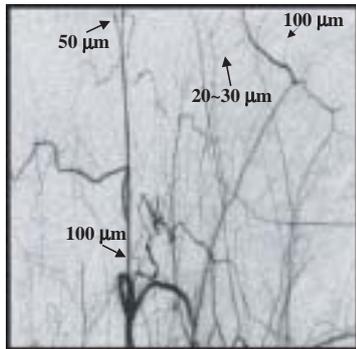


Fig. 1. (a) Tumor growth and modification of microvasculature with time. (b) Modification of tumor microvasculature according with time on microangiographic images.

new blood vessels (angiogenesis). The results obtained using the present technique are in close agreement with those obtained by molecular biological and histological analyses, indicating that the present technique is promising. In the present study, a rat tumor was transplanted, but with the present experimental system, human-derived tumor cell lines can be heterogeneously transplanted. Progress in human chemotherapy, molecular target therapy and radiotherapy will be realized with further technical advances.



field of view : 9.5×9.5 mm / 1024×1024 pixel

Fig. 2. Microangiographic image of rat superficial inferior epigastric vessels.

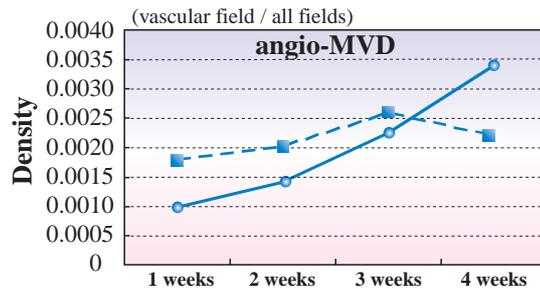
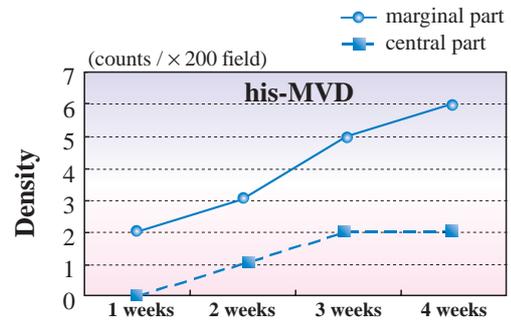


Fig. 3. Histological (his-) and angiological (angio-) microvessel densities (MVD) with tumor growth.

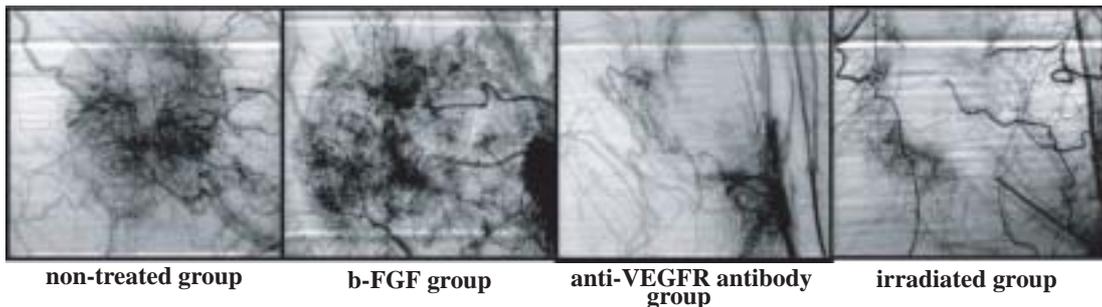


Fig. 4. Typical microangiographic changes recognized in tumors of the non-treated and treated groups (four weeks).

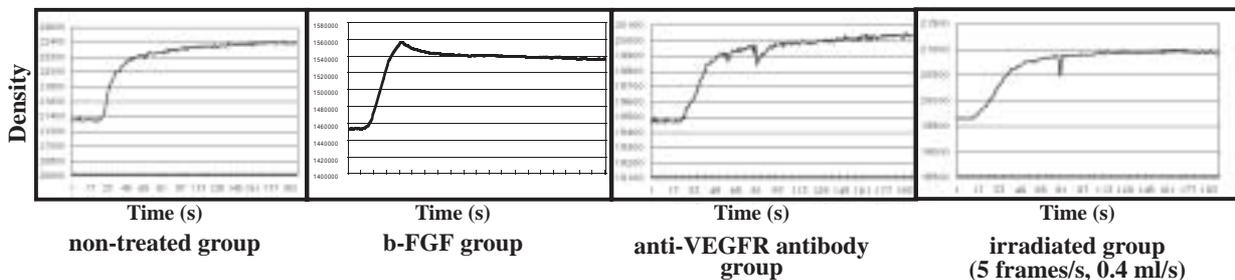


Fig. 5. Microangiographic time-density curves in tumors of non-treated and treated groups (four weeks).

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## References

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