As cancer chemotherapy damages rapidly growing cells, not only tumour cells but also normal growing cells such as bone marrow and intestinal cells are damaged. These normal cell damages appear as side effects. Chemotherapy may also damage angiogenic endothelial cells, since these cells are growing cells, although this effect had not been well noticed since this effect is included in main effect of the antitumour agent.

Browder *et al.* determined an appropriate administration scheduling for cancer treatment [93]. This schedule was characterised by low dose injection at short intervals, and named 'antiangiogenic schedule', because they observed apoptosis of angiogenic endothelial cells prior to tumour cell killing. The antiangiogenic schedule showed marked suppression of angiogenesis, since a shorter treatment-free period might not allow reconstruction of new blood vessels. It was also demonstrated that this enhanced antiangiogenic effect brought marked suppression of tumour growth. It was confirmed in various kinds of cancer models. Additionally, this schedule could be applied to drug-resistant tumour and p53-deficient tumour cell lines, since the mechanism was based on disruption of angiogenic vasculature [94]. Furthermore, administration of liposomal formulation of cytotoxic agent in antiangiogenic schedule showed more enhanced antitumour effect compared with free formulation of it: liposomal formulation might allow sustained drug release. Combinational administration of both cytotoxic agent and angiogenic inhibitor in antiangiogenic schedule also showed more enhanced antitumour effect compared with administration of cytotoxic agent alone [93].

Kerbel *et al.* reported that repeated low-dose administration (metronomic dosing) showed potent therapeutic effect and prolongation of survival time of mice in various cancer models [94]. They also demonstrated the effectiveness of metronomic dosing in drug-resistant cancer model. This therapeutic scheduling is now waiting to be taken into clinical cancer chemotherapy [95].

4.5 Antiangiogenic photodynamic therapy

Photodynamic therapy (PDT) promises potent efficacy against neoplastic and abnormal tissues such as tumour tissue. PDT uses a combination of photosensitiser, such as porphyrin, chlorin, or phthalocyanine derivatives, and tissue-penetrating visible laser light. In brief, laser light promotes the photosensitiser into an excited state, and when it comes back to ground state, activated oxygen, such as singlet oxygen, is generated by interaction with oxygen. Activated oxygen then directly kills tumour cells [96]. As laser irradiation can be localised around the tumour, severe side effects that are usually observed in chemotherapy can be avoided. Benzoporphyrin derivative monoacid ring A (BPD-MA) is a second generation of photo-sensitiser, and their liposomal formulation is commercialised as Visudyne™ (Quadra Logic Technologies/Novartis) for the treatment of AMD in which uncontrolled angiogenesis occurs. Specific laser irradiation at angiogenic site causes eradication of angiogenic endothelial cells, resulting in disruption of angiogenic vasculature [97]. Based on this idea, the authors established a novel photodynamic cancer therapy targeting to angiogenic vasculature, namely antiangiogenic PDT. This therapy also targets on the growing angiogenic endothelial cells of newly formed vessels.

The authors previously determined antiangiogenic scheduling of photodynamic therapy for cancer therapy, which performed laser irradiation 15 min after administration of photosensitiser (15-min PDT). In general, laser irradiation is performed 3 – 5 h after administration of BPD-MA because the photosensitiser highly accumulates in tumour tissue at those times. However, at earlier times such as 15 min after administration, the photosensitiser mainly exists in bloodstream and may be incorporated in angiogenic endothelial cells rather than in tumour cells. In fact, antiangiogenic PDT scheduling (15 min PDT) effectively damaged angiogenic vasculature compared with 3-h PDT by use of dorsal air sac model. Furthermore, in a therapeutic experiment, 15-min PDT using liposomal BPD-MA effectively suppressed tumour growth and showed prolonged survival time of solid tumour-bearing mice [98,99].

To enhance electrostatic interaction of liposomal BPD-MA with angiogenic endothelial cells, the authors used positively charged liposomes as a carrier for BPD-M, because the surface of endothelial cells are negatively charged [100]. For this purpose we used polycation liposomes (PCLs): liposomal surface was coated with polyethylenimine. BPD-MA-encapsulated PCLs showed strong binding to endothelial cells and enhanced cytotoxic effect against endothelial cells after laser irradiation *in vitro* [101,102]. Corresponding to this *in vitro* data, BPD-MA-encapsulated PCLs showed potent therapeutic effect such as tumour regression and prolonged survival time in solid tumour-bearing mice after irradiation of laser 15 min after administration [103]. Furthermore, it has been clarified that enhanced tumour regression by BPD-MA PCL-mediated PDT depends on disruption of angiogenic vasculature. These observations indicate that antiangiogenic PDT is expected to be efficient cancer therapy compared with traditional PDT.

5. Conclusion

Tumour angiogenesis is a critical event for solid tumour growth, and research has been carried out to develop inhibitors for blocking certain step in angiogenesis. Antiangiogenic therapy is thus proposed. There are many good reviews on antiangiogenic therapy which aims to inhibit certain angiogenic processes such as VEGF-mediated signalling and MMP-related ECM degradation, and so on [104]. Recently, it has become noticed that cancer chemotherapy damages angiogenic endothelial cells as well as tumour cells, because angiogenic endothelial cells are growing cells, such as tumour cells. And new strategy for antiangiogenic therapy is proposed, which aims to damage pre-formed neovessels. In this review, the authors described three different approaches;

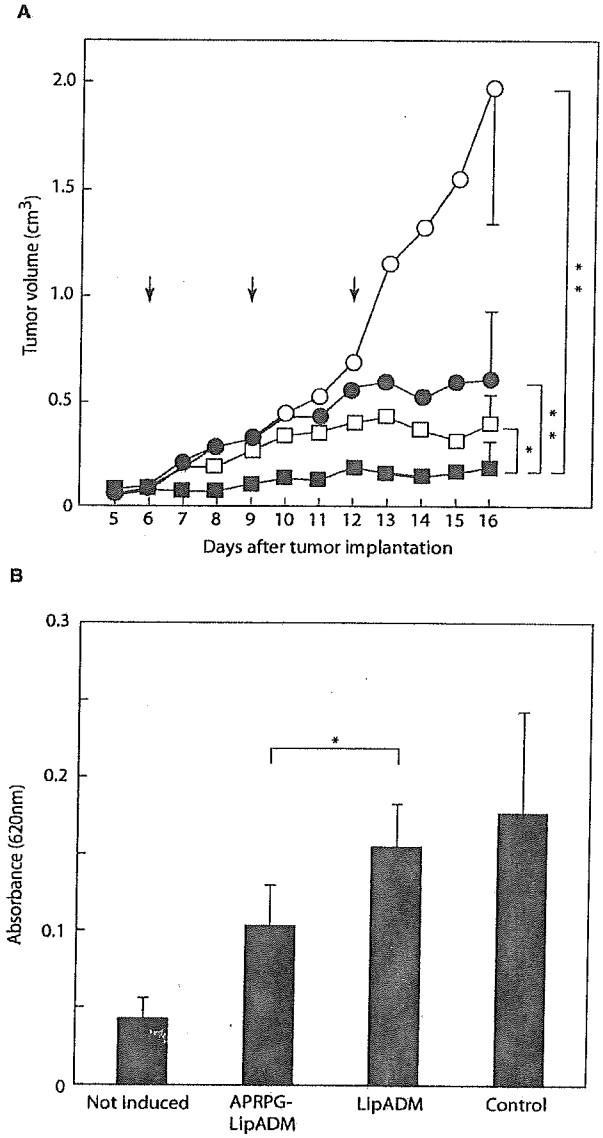

Figure 3. Antitumour (antineovascular) therapeutic experiment with adriamycin-encapsulated liposomes. ADM-encapsulated liposome was prepared by a modification of the remote-loading method, and the encapsulation efficiency was > 90% throughout the experiment. **A)** Suppression of tumour growth by APRPG-LipADM in Meth A sarcoma-bearing mice. ADM encapsulated in control liposome (white square = LipADM) or in liposome-modified with stearoyl APRPG (black square = APRPG-LipADM) (10 mg/kg as ADM), ADM alone (black circle = 10 mg/kg), or 0.3M glucose solution (white circle = control) were injected intravenously into tumour bearing BALB/c mice (n = 6) at days 6, 9 and 12 after implantation of Meth A sarcoma cells. The size of the tumour and body weight of each mouse were monitored daily. Tumour volume was calculated using the formula 0.4 (a x b2), where "a" was the largest and "b" was the smallest diameter of the tumour. Variance in a group was evaluated by the F-test, and differences in mean tumour volume were evaluated by Student's t-test. Data are presented as mean tumour volume and SD. SD bars are shown only for the last points for the sake of graphic clarity. Arrows show the day of treatment. Significant differences are indicated (*, $p < 0.05$; **, $p < 0.01$). **B)** Suppression of in vivo angiogenesis by APRPG-LipADM. For assay of antineovascular activity, a chamber ring loaded with Colon 26 NL-17 cells (1 x 107 cells/ring) was dorsally inoculated into five-week-old BALB/c male mice. At 2 days after inoculation, LipADM, APRPG-LipADM (10 mg/kg as ADM), or 0.3M glucose solution were injected intravenously into DAS model mice. At 4 days after inoculation, 1% Evans Blue solution was injected intravenously into the mice. After 1 min, they were sacrified and the pigment in the skin attached to the ring was extracted for the measurement of absorbance at 620 nm. The data represent an absorbance of the pigment at angiogenic site and significant difference from the LipADM is indicated (*, $p < 0.05$).

ADM: Adriamycin; APRPG: Ala-Pro-Arg-Pro-Gly; DAS: Dorsal air sac; LipADM: Liposome adriamycin; PRP: Platelet-rich plasma; SD: Standard deviation.

firstly one aims to deliver cytotoxic agents to angiogenic endothelial cells, secondly to function cytotoxic agents more to angiogenic endothelial cells than to tumour cells by altering administration scheduling of the agents, and thirdly to damage angiogenic endothelial cells by use of appropriate scheduling and appropriate carrier of photosensitiser in PDT. To clarify the difference of this strategy from conventional antiangiogenic therapy, the authors named this as 'anti-neovascular therapy (ANET)'.

Inhibition of angiogenic process is a promising approach, however, it sometimes brings only tumour dormancy. Moreover, most of the angiogenesis of these inhibitors need an abundant dose and frequent administration to suppress tumour growth. Selective but not drastic activities of these inhibitors may make clinical studies difficult. On the contrary, ANET aims to disrupt angiogenic vasculature by delivering cytotoxic agent to angiogenic vessels. Therefore, ANET is expected not only to suppress tumour growth, but also to eradicate tumour cells through complete cut-off of oxygen and nutrients. In general, cancer chemotherapy is accompanied with strong side effects and acquired drug resistance. Therefore, drug delivery systems which selectively deliver the drugs to the target tumour are awaited. Recently, vascular targeting has become a focus of interest, since certain drugs or drug carriers first meet neovasculature before extravasation in the tumour. In particular, targeting of a tumour angiogenic vasculature is promising for cancer treatment since these vessels have properties different from those of the pre-existing systemic vasculature. Furthermore, angiogenic endothelial cells are growing cells, and are effectively damaged by antitumour drugs if the drugs are appropriately delivered to or functioned on the neovessel cells. In this review, the authors have shown that the direct eradication of angiogenic endothelial cells is actually more potent to eradicate tumours than the direct damaging of tumour cells by angiogenic vasculature-targeting 'ANET'. ANET including metronomic-dosing chemotherapy and antiangiogenic PDT would be a hopeful treatment modality for cancer patients.

6. Expert opinion

Since cancer became one of the higher fatality diseases in developed countries, various kinds of anticancer agents have been developed for cancer therapy. Although they show effective direct cytotoxicity against tumour cells, most of them accompany severe side effects, such as myelosuppression, because they also damage some growing normal cells. This problem is mainly caused by low selectivity of the drugs, since most anti-cancer agents show their dramatic cytotoxicity in a cell cycle-dependent manner. Due to this problem, clinical MTD of anticancer agents often fails to show enough therapeutic efficiency. Additionally, acquirement of drug resistance in tumour cells sometimes causes difficulty in cancer therapy. For these reasons, development of novel cytotoxic agents becomes more difficult. Thus, introduction of drug delivery system (DDS) technology and a novel approach for cancer therapy are now expected.

Since Folkman and co-workers stated the importance of angiogenesis in tumour growth in the earlier 1970s [105] and discovered angiostatin in 1996 [49], angiogenesis in cancer research has been considered. Up till now, the angiogenic processes and involvement of angiogenic factors and signal transducing molecules have been elucidated. According with this interest, cancer therapy targeting angiogenesis has been focused. In the present review, the authors have firstly introduced various kinds of targeting molecules for antiangiogenic therapy and their antitumour effect. As a result, it has been clarified that angiogenesis is processed with complex stages where angiogenic endothelial cells play an important role. In brief, angiogenesis initiates with interaction of angiogenic factors with their receptors, following with signal transduction, endothelial cell proliferation, migration, invasion, and tube formation. Antiangiogenic therapy aims to inhibit one or several steps of angiogenesis and subsequently to suppress tumour growth. However, it is questionable whether: an injectable dose could completely suppress tumour angiogenesis; suppression of angiogenesis leads to complete eradication of tumour cells; whether antitumour effect lasts long-term; and whether it is applicable for any stage or various kinds of tumours. In fact, some cases are reported: natural angiogenic inhibitor, endostatin showed effective inhibition of angiogenesis in early stage of tumour but not shown in late stages. Additionally, although various kinds of antiangiogenic agents have been developed, some doses do not show satisfactory antitumour effect in clinical study. One of the reasons for this is based on the alternative functions of a variety of pro-angiogenic factors in various stages of angiogenesis. To overcome this problem, a novel approach in antiangiogenic therapy has been expected.

Since conventional cytotoxic anticancer drugs target angiogenic endothelial cells as well as other growing cells, the authors developed one of a novel antiangiogenic therapy, ANET. ANET includes angiogenic vasculature-targeting chemotherapy, metronomic-dosing therapy, and antiangiogenic photodynamic therapy. Angiogenic vasculature-targeting chemotherapy was achieved with active-targeting tools and DDS technology: anticancer drug-encapsulating liposomes modified with a peptide specifically bound to angiogenic endothelial cells were used. This liposomal anti-cancer drug suppressed tumour growth in a tumour-bearing mice model. Furthermore, usage of DDS technology decreased side effects by lowering administration dose of cytotoxic agents and by altering biodistribution of the agents because of the targeting effect. Metronomic-dosing chemotherapy aims to shift the action site of cytotoxic agents from tumour cells to angiogenic endothelial cells with continuous low-dose administration scheduling and shows potent anti-tumour effect against various cancers including drug-resistant cancer. Antiangiogenic PDT, which also shifts

the target from tumour cells to angiogenic endothelial cells, shows efficient antitumour activity with reduced side effects. Thus, these strategies promise complete cancer cure with overcoming the conventional problems such as severe side effects and drug selection. We hope that ANET will be applied in clinical cancer therapy in the future.

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