Vascular disrupting therapy-induced mobilization of circulating endothelial progenitor cells

Antivascular therapies are currently intensively studied approaches in clinical oncology. Low molecular weight vascular disrupting agents (VDA) aim to cause rapid and selective shutdown of the established tumor vasculature, leading to massive cancer cell death in the central areas of tumor [1]. However, a common feature of VDA that is poorly understood is the sparing of a relatively intact viable rim at the periphery of the tumor. This viable rim leads to rapid regrowth of tumors and consequently poor responses to VDA when given as a single agent [1]. Combination therapy with chemo- or radiotherapy has produced promising responses in preclinical models and is currently being evaluated in early clinical developments.

Recent studies in mice have demonstrated that circulating bone marrow-derived endothelial progenitor cells (CEP) can

Figure 1. Flow cytometric detection of CEP. Panels 1A and 1B show results in a representative sample (drawn from patient 5 at day 7). (1A) Analysis of the control PE (CD45-FITC/mouse IgG1 PE/CD34-APC/7AAD) tube. (1B) Analysis of the test (CD45-FITC/KDR PE/CD34-APC/7AAD) tube. CD45<sup>+</sup>CD34<sup>+</sup>KDR<sup>+</sup>7AAD<sup>–</sup> events defined as CEP are highlighted in bold.
home to ischemic sites, differentiate into endothelial cells and restore organ vascularization [2]. CEP are mobilized from the bone marrow by tumor-derived cytokines and their contribution to tumor neoangiogenesis in mouse models ranges from 2 to 50% [3, 4]. In humans, whether CEP have a significant participation in tumor neoangiogenesis remains unclear. An elegant study in mice recently demonstrated that VDA treatment in tumor-bearing animals induced an acute mobilization of CEP which then home to the peripheral tumor rim [5]. Disruption of this CEP burst by anti-angiogenic drugs or genetic manipulation resulted in enhanced antitumor VDA efficacy.

We took advantage of an ongoing phase I trial combining the antivascular agent AVE8062 (Sanofi-Aventis) and cisplatin to evaluate CEP in cancer patients. We monitored CEP levels in three blood samples drawn at baseline, day 3 and day 7 in five patients. CEP were measured in ficoll gradient mononuclear cells (MNC) enriched fractions by four-colour flow cytometry using CD45-FITC, VEGFR2(KDR)-PE, CD34-APC monoclonal antibodies and a viability dye (7AAD) to eliminate background noise occasioned by dead cells. CEPs identified as CD45dim CD34+ VEGFR2(KDR)+ 7AAD− events were defined as the rare fraction of circulating progenitor cells (CD45dim CD34+ VEGFR2(KDR)+ 7AAD− events) that expressed the VEGFR2(KDR) receptor [5, 6]. Preliminary experiments have shown that CD45dim CD34+ VEGFR2(KDR)+ 7AAD− cells were in the vast majority also positive for the CD133 marker.

MNC were stained according to standard methods. MNC were distributed into control and test tubes and treated with FcR blocking reagent (Miltenyi Biotec, Germany). The monoclonal antibodies and reagents used were CD45-FITC (clone T29/33, DakoCytomation, Denmark), CD34-APC (clone BIRMA-K3, DakoCytomation), KDR-PE (clone 89106, R & D Systems, MN, USA) and 7AAD (BD Biosciences, Belgium). Control tubes included isotypic and fluorescence minus one (FMO) controls for each of the fluorochromes. To accurately measure the background noise and precisely adjust the gates, a control PE tube including a mouse IgG1 PE reagent (CD45-FITC/mouse IgG1 PE/CD34-APC/7AAD) was performed. Cells were analysed on a FACSCalibur (BD Biosciences).

At baseline, CEP values ranged from 0.1 to 7.4% of circulating progenitor cells. An increase was observed on day 3 for three patients, with CEP values shifting from 7.4 to 30.9% for patient 1, from 0.4 to 4.9% for patient 2 and from 0.1 to 11.2% for patient 4. In these patients, CEP values decreased at day 7 but remained notably high in patient 1 (23.1%). For the two other patients, the CEP peak was observed at day 7, with values shifting from 0.5% at baseline to 2.7% at day 7 in patient 3 and from 0.4% to 3.9% in patient 5 (Figure 1). CEP levels increased at day 3 by a factor of 4, 16 and 37 for patients 1, 2 and 4, respectively, and by a factor of 5 and 10 for patients 3 and 5 at day 7. Preliminary experiments have shown that of the CD45dim CD34+ VEGFR2(KDR)+ 7AAD− cells, the vast majority were also positive for the CD133 marker.

We show for the first time that VDA-induced CEP mobilization, previously described in mice models, is also present in humans. The acute tumor insult induced by VDA results in sudden and severe hypoxia, similar to that of pathological tissue ischemia in murine models [2]. As observed in these models, vascular trauma could result in an upregulation of angiogenic factors that could promote the mobilization of bone marrow-derived endothelial progenitors and their possible homing to damaged tumor vessels. Our results strongly support the rationale to combine VDA with anti-angiogenic therapies. Furthermore, whether VDA is combined with chemo- or anti-angiogenic therapy, our results emphasize the importance of adding an anticancer agent to a VDA in an appropriate timing sequence, in order to counteract the salvage effect of CEP.

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