Contribution of bone marrow–derived endothelial cells to human tumor vasculature

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It has been shown that bone marrow–derived stem cells can form a major fraction of the tumor endothelium in mouse tumors. To determine the role of such cells in human tumor angiogenesis, we studied six individuals who developed cancers after bone marrow transplantation with donor cells derived from individuals of the opposite sex. By performing fluorescence *in situ* hybridization (FISH) with sex chromosome–specific probes in conjunction with fluorescent antibody staining, we found that such stem cells indeed contributed to tumor endothelium, but at low levels, averaging only 4.9% of the total. These results illustrate substantial differences between human tumors and many mouse models with respect to angiogenesis and have important implications for the translation of experimental antiangiogenic therapies to the clinic.

Several studies in mice have shown that circulating endothelial cells derived from bone marrow contribute to tumor angiogenesis[1,2]. The extent of the contribution varies from 0% to close to 100%, depending on the tumor type, host and stage of tumorigenesis[3]. To date, only a few studies have attempted to assess the importance of bone marrow–derived endothelial cells in human tumor neovascularization and to determine whether any of the mouse models mimic the human state[4]. The paucity of human data on this topic probably results from the difficulties in obtaining appropriate samples and the technical challenges involved in analyzing them.

An extensive search of databases at the major transplant centers in the United States allowed us to identify a set of samples from individuals who developed cancers after bone marrow transplantation with donors of the opposite sex. The cancers developed 15 months to 15 years after bone marrow transplantation and represented several human primary tumor types, including lymphomas, carcinomas and sarcomas (**Supplementary Table 1** online). Multicolor FISH with X- or Y-chromosome–specific probes was combined with fluorescent antibody staining to unambiguously identify donor cells in paraffin-embedded sections (**Supplementary Methods** online)[5,6]. Endothelial cells were stained with a well-characterized antibody against von Willebrand factor (vWF), and leukocytes were identified with CD45 and CD44 antibodies. The use of antibodies specific for the leukocyte antigens was essential to avoid false-positive signals arising from leukocytes closely adherent to vessels. Endothelial cells (vWF^+ $CD45^-$) were categorized as donor cells if they contained one Y chromosome FISH signal (in male-to-female transplants) or two

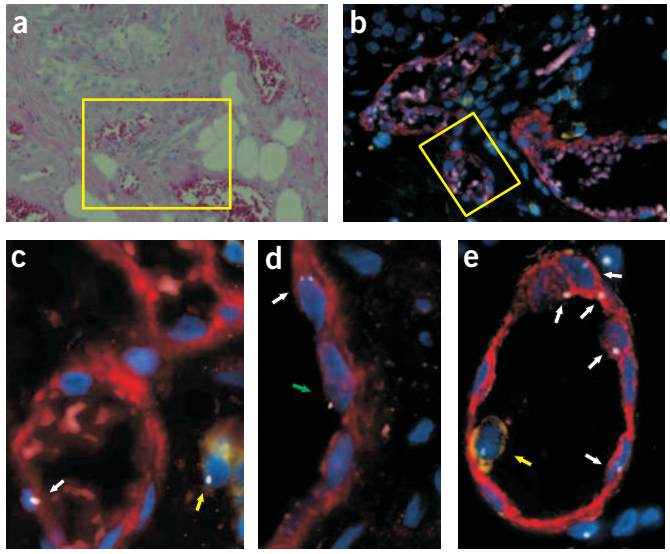

Figure 1 Blood vessels of human tumors were analyzed by FISH with sex chromosome–specific probes (white) in conjunction with fluorescent antibody staining. Anti-human von Willebrand Factor (red) stained endothelial cells, and anti-human CD45 (yellow) stained leukocytes. Yellow arrows point to CD45-positive cells with a Y chromosome signal (**c**–**e**). Staining was captured in five different fluorescent channels using a Leica DMRA2 microscopic workstation and the Leica CW4000 FISH imaging software. (**a**–**c**) Section of a female thyroid cancer after male bone marrow transplant. (**a**) Low magnification image of tumor section stained with hematoxylin and eosin. Large blue nuclei indicate tumor cells. Yellow box outlines region of higher magnification in **b**. (**b**) Multichannel fluorescent image of an adjacent section. Yellow box outlines vessel of interest examined in **c**. (**c**) Blood vessel with one endothelial cell showing a Y chromosome signal (white arrow). (**d**) Portion of a vessel of a male glossal mucoepidermoid carcinoma after bone marrow transplantation from a female donor, containing an endothelial cell with two X chromosome signals (white arrow). Another nucleus with a single X chromosome (green arrow) can also be seen, presumably arising from male recipient cells. (**e**) Vessel of a male with colon cancer who had not undergone bone marrow transplantation, showing many endothelial cells exhibiting FISH signals for the Y chromosome (white arrows). Magnification, 200 in **a**, 400 in **b**, 630 in **c**–**e**.

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Table 1 Combined immunofluorescence and FISH data

Sample	Patient sex	Donor sex	Number of vessels analyzed	vWF$^+$ CD45$^-$ cells	BMDC	BMDC (percent)	BMDC (percent) normalized[a]
Spindle cell sarcoma, head and neck	F	M	58	215	1	0.5	1.0
Hodgkin lymphoma	F	M	37	219	11	5.0	12.1
Mucoepidermoid carcinoma, submandibular	F	M	38	192	7	3.6	7.0
Thyroid carcinoma	F	M	95	752	4	0.5	1.1
Osteogenic carcinoma	M	F	36	293	3	1.0	4.1
Mucoepidermoid carcinoma, glossal	M	F	11	94	1	1.1	4.0

BMDC, bone marrow–derived cells. [a]The detection frequency of endothelial cells with a donor-specific FISH signal was normalized to the fraction of endothelial cells in matched tumor types in which the relevant sex chromosome could be detected (**Supplementary Table 2** online).

X chromosome FISH signals (in female-to-male transplants) in their interphase nuclei (**Fig. 1**).

As a positive control, we first evaluated 1,648 vWF-positive CD45-negative cells derived from 152 vessels in 11 tumors of male and female individuals who had not undergone bone marrow transplantation (**Fig. 1e** and **Supplementary Fig. 1** and **Supplementary Table 2** online). Samples were selected to correspond with the tumor types of the transplant patients. This allowed us to control for any unexpected differences in hybridization efficiency or size of endothelial nuclei between different tissues. The fraction of endothelial cells from male samples containing Y chromosomes in their nuclei averaged 50% (range, 42–56%). These numbers were consistent with the fact that the paraffin sections analyzed were 4 m thick and therefore only included a fraction of the volume of each nucleus. Because all sections in the transplant patients were of the same thickness, the actual percentage of bone marrow–derived endothelial cells in the tumor vasculature could be estimated by multiplying the observed number of donor sex chromosome–positive, vWF$^+$ CD45$^-$ cells by a 'normalization factor' derived from these positive-control experiments (**Supplementary Table 2** online).

We then analyzed the vessels of the six transplant individuals described above. At least one blood vessel containing endothelial cell(s) derived from the donor bone marrow was observed in each individual (**Fig. 1a–d**). In total, 1,765 vWF$^+$ CD45$^-$ cells were evaluated and 27 cells containing FISH signals indicating their donor origin were detected. The percentage of bone marrow–derived endothelial cells in the tumor vasculature averaged ~4.9% and ranged from 1% to ~12% (**Table 1**). Bone marrow–derived cells were randomly distributed throughout the tissue of the samples analyzed, and we never found more than two such cells in any vessel.

Several studies have suggested that bone marrow–derived stem cells contribute differentiated cells to nonhematopoietic tissues through cell fusion[7,8]. We therefore inspected vessels of three of the transplant individuals with FISH probes for both the X and Y chromosomes simultaneously. We were able to identify a total of six examples of bone marrow–derived endothelial cells in these individuals and found that each contained a normal diploid copy number of sex chromosomes. This finding indicated that cell fusion was not generally involved in the differentiation of bone marrow–derived cells to the tumor endothelial cell lineage.

As it is conceivable that the residual host endothelial progenitors of individuals with bone marrow chimerism may compete with the donor-derived cells for the incorporation into the tumor vessel wall, we determined the degree of marrow chimerism using FISH. We applied Y and X chromosome–specific probes to all six transplant individual samples and identified 825 bone marrow–derived cells (CD44$^+$ CD45$^+$). For all but one sample, no host-derived bone marrow cells could be found (**Supplementary Table 3** online). In the osteogenic sarcoma sample, a small number (5% of 80 cells analyzed) of host bone marrow–derived CD44$^+$ CD45$^+$ cells were detected. The low extent of host marrow cells available for incorporation into tumor vessels makes it extremely unlikely that competition between host and donor accounts for the low fraction of marrow-derived endothelial cells we observed. It is possible, though not likely, that in transplant individuals endothelial progenitor chimerism differs from hematopoietic chimerism, and further studies to quantify the contribution of endothelial chimerism in humans in a variety of tumor types and stages of growth should prove informative[9].

The contribution of circulating bone marrow stem cells to tumor endothelium has important implications for gene therapy approaches using bone marrow stem cells as delivery agents[10,11] as well as for more conventional antiangiogenic drugs[12]. Our results show that bone marrow stem cells definitely contribute to tumor angiogenesis in diverse human tumor types, but that this contribution is relatively small. This level is more similar to that observed in autochthonous tumors in transgenic mice than in transplanted tumors in mice[13,14]. Notably, the level we observed in tumors is very similar to that recently observed in normal human tissues[15].

Note: Supplementary information is available on the Nature Medicine website.

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COMPETING INTEREST STATEMENT

The authors declare that they have no competing financial interests.

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