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Tumorigenesis and Neoplastic Progression

Fetal Microchimeric Cells Participate in Tumour Angiogenesis in Melanomas Occurring during Pregnancy

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Melanoma is a major malignancy in younger individuals that accounts for 8% of all neoplasias associated with gestation. During pregnancy, a small number of fetal cells enter the maternal circulation. These cells persist and then migrate to various maternal tissues where they may engraft and differentiate, particularly if there is organ damage, adopting the phenotype of the host organ. To understand the relationship between melanoma and pregnancy, we analyzed these tumors in both humans and mice. Fetal cells were detected in 63% of human primary melanomas versus 12% in nevi during pregnancy ($P = 0.034$) and in 57% of B16 melanomas in pregnant mice but never in normal skin ($P = 0.000022$). More than 50% of these fetal cells expressed the CD34, CD31, or von Willebrand factor endothelial cell markers. In addition, the Lyve-1 lymphatic antigen was expressed by more than 30% of fetal cells in mice. In conclusion, we show that melanomas during pregnancy frequently harbor fetal cells that have an endothelial phenotype. Further studies are needed to assess whether the fetal contribution to lymphangiogenesis may alter the prognosis of the maternal tumor. ***(Am J Pathol 2009, 174:630–637; DOI: 10.2353/ajpath.2009.080566)***

The incidence of melanoma during gestation ranges from 2.8 to 5 per 100,000 pregnancies[1] accounting for ~8% of all cancers diagnosed in pregnant women.[2,3] In western countries, melanoma is the most common malignancy in pregnant women younger than 30 years of age. Since the incidence of melanoma has grown substantially during past decades and because this tumor mainly develops in 20- to 40-year-old patients, its occurrence during gestation is expected to rise.

During pregnancy, fetal cells enter the maternal circulation.[4,5] These cells may persist in maternal tissues decades after delivery,[6,7] and include hematopoietic or mesenchymal stem cells.[6,8] They may engraft in a variety of maternal tissues especially if there is specific organ damage.[9,10] In these tissues, fetal cells adopt the phenotype of the affected maternal organ. These observations combined with similar results in animal studies clearly suggest a specific homing of fetal progenitor cells to maternal effected tissues and their differentiation into the phenotype of the maternal tissue.[9–13] The presence of fetal cells has been found in many situations including

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cancers.[14] More recently, we have shown that during pregnancy, fetal endothelial progenitor cells might be recruited to maternal inflammatory skin and form functional blood vessels.[15] Based on the fact that fetal cells may participate in the remodeling of maternal tissues and given the importance of angiogenesis in the progression of melanoma, we aimed to evaluate the presence and the phenotype of fetal cells in melanomas during gestation in humans as well as animal models.

Materials and Methods

Mice with Melanoma during or Outside Gestation

Wild-type C57BL/6 (B6) female mice (8 weeks old) were bred to males transgenic for the enhanced green fluorescent protein under the control of the chicken β-actin promoter (named EGFP$^+$), kindly provided by Dr. Masaru Okabe (Osaka University, Osaka, Japan).[16] All mice were treated in accordance with the institutional guidelines for the care of experimental animals.

For tumor implantation, 1 million B16-BL6F10 cells were injected intradermally in the right flanks of all female mice 5 days after mating. At day 16 after inoculation, mice were sacrificed whether pregnant or not. Pregnancy was easily observed clinically but also confirmed by dissecting the uterus. All fetuses were assessed under UV light (365 nm) to ensure that each pregnant mouse had at least one transgenic fetus. Injected primary tumors, metastases, and healthy skin from the ear were collected and frozen in liquid nitrogen with or without prior fixation in 4% formaldehyde for 2 hours. Nonpregnant mice were used as controls.

Pregnant Women with Melanoma or Nevi

Clinicopathological records of six different hospitals in France were retrospectively reviewed in accordance with local committee and European Union ethical regulations for patient's information and consent during biomedical research. Women with malignant melanoma or benign nevi were identified and included if i) the primary tumor had been excised during pregnancy or in the 6 months after delivery; ii) women carried or delivered a male fetus during that pregnancy; and iii) additional formalin-fixed, paraffin-embedded material was available. None of the patients had any history of blood transfusions, organ transplantation, chemotherapy, or radiotherapy. For each woman, archived paraffin-embedded surgical specimens were collected. Histological and clinical parameters such as Breslow index (tumor thickness), Clark's level, and the presence of regional lymph node invasion at the time of diagnosis were recorded. For controls, archival specimens of benign melanocytic naevi from women pregnant with a male fetus or having given birth to a son during the past 6 months were also collected. Finally, we also obtained seven skin biopsy specimens of polymorphic eruptions of pregnancy from patients that were pregnant with female fetuses.

Fluorescent in Situ *Hybridization (FISH)*

FISH was performed on paraffin-embedded human tissue sections as previously described[17] using the CEP X SpectrumOrange/Y SpectrumGreen DNA probe kit (Vysis, Abbott, Abbott Park, IL). Similarly, X and Y chromosome FISH was performed on 4% formaldehyde-fixed cryosections from mouse melanomas. We used fluorescein isothiocyanate-labeled Y chromosome and rhodamine-labeled X chromosome whole paint Starfish probes (Cambio Ltd., Cambridge, UK). Slides were examined under a fluorescence microscope, Axioplan 2 (Zeiss, Göttingen, Germany), and photographed with a Genicon (San Diego, CA) software program. Each slide was scanned and analyzed if more than 75% of the nuclei contained the X hybridization signals. To be considered as male within the tissue sections, a cell should show a blue nucleus containing only two different-colored FISH signals: a green-colored fluorescent signal of a Y chromosome and a red-colored fluorescent signal of a X chromosome. The number of male fetal cells per section was noted and the total number of nuclei on each section was estimated. Slides were scored blinded to the histology of the sample (naevus or melanoma). As a control for the FISH quality, polymorphic eruptions of pregnancy specimens from women pregnant with female fetuses were also analyzed for the presence of male cells.

Combined FISH with Immunostaining on Sections from Humans

Immuno-FISH was performed on human tissue sections as previously described.[18] The primary antibodies were monoclonal mouse anti-human CD45, CD31, or CD34, monoclonal mouse anti-human cytokeratin AE1/AE3, and mouse anti-human HMB-45 monoclonal antibodies (1:50; Biocompare, South San Francisco, CA). Staining was revealed by Envision + peroxidase kit (Envision kit; DakoCytomation, Glostrup, Denmark) for CD45, CD31, or CD34. For cytokeratin and HMB-45 labeling, we used immunofluorescence with secondary goat anti-mouse antibody labeled with fluorescein (Jackson ImmunoResearch, West Grove, PA). All slides were counterstained with 4′,6-diamidino-2-phenylindole (DAPI).

Single and Double Immunostaining on Mouse Tissues

Immunofluorescence staining was performed on animal sections as described. Briefly, after permeabilization (Triton X-100), sections were blocked using 20% normal goat serum (DakoCytomation). Primary antibodies used were the rabbit anti-EGFP polyclonal antibody (1:100; Chemicon International, Temecula, CA), rabbit polyclonal anti-von Willebrand factor (1:800; Abcam, Cambridge Science Park, UK), rabbit polyclonal antibody anti-mouse Lyve-1 (1:200, Abcam), rat anti-mouse CD45 (1:10; BD Pharmingen, Franklin Lakes, NJ). The secondary antibodies were the goat anti-rabbit IgG labeled with fluorescein isothiocyanate, goat anti-rabbit IgG labeled with

Table 1. Detection of Fetal Cells in Human and Mouse Melanomas

	Human*	Mouse†
Melanoma	10/16	13/23
Control	1/8	0/23
P‡	0.033	0.000022

Controls: *naevi, †normal skin, ‡Fisher's exact test.

Texas Red, goat anti-rat IgG labeled with Texas Red (1:100, Jackson ImmunoResearch). Nuclei were counterstained with 0.3 μg/ml DAPI. Slides were then observed under a fluorescence microscope (Leica, Deerfield, IL) with a QImaging digital camera (Media Cybernetics, Silver Spring, MD). Mouse samples were analyzed for the presence of EGFP$^+$ cells blinded as to their pregnancy status. In addition to anti-EGFP immunofluorescence, all samples were also examined for the spontaneous fluorescence of EGFP.

Confocal Laser-Scanning Microscopy

We used confocal laser-scanning microscopy to confirm morphology as well as phenotype of fetal EGFP$^+$ cells in maternal tissue. Confocal microscope analysis was performed using the TCS SP2 Leica (Lasertechnik GmbH, Vienna, Austria), equipped with a ×40 objective. For GFP excitation, an argon-krypton ion laser adjusted at 488 nm was used, 568 nm for Texas Red or propidium iodide excitation and 370 nm for DAPI excitation. For each

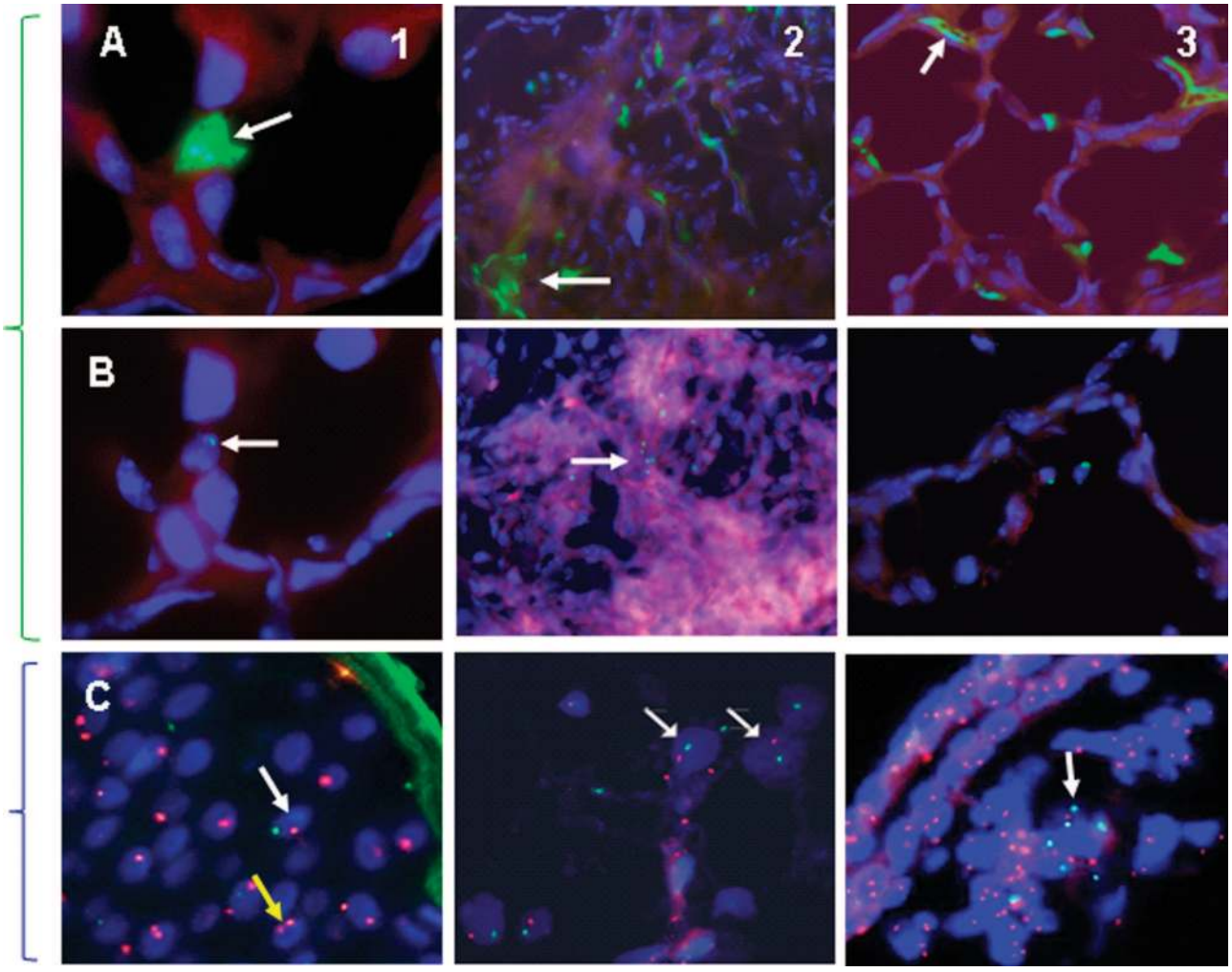

Figure 1. Fetal microchimeric cells home to melanomas during pregnancy. **A** and **B:** Presence of fetal cells in B16-BL6 mouse melanoma. B16-BL6 tumors were obtained 16 days after injection from pregnant mice bearing EGFP transgenic fetuses. Sections were counterstained with DAPI (blue) to identify nuclei and labeled with anti-EGFP antibody (**A**) followed by FISH using a Y chromosome paint (**B**). **A:** Photomicrographs showing immunofluorescence staining with anti-EGFP antibody. **A1:** A fetal GFP$^+$ cell; **A2:** groups of EGFP$^+$ cells reside at the periphery of the tumor; **A3:** fetal cells (**arrow**) with the morphology of blood vessels were localized in the walls of septa surrounding the tumor. **B:** Photographs showing male fetal cells containing one Y chromosome inside the nucleus with intact borders located in mouse tumors. **B1:** The EGFP$^+$ cell in **A1** is a male cell (**white arrow**). Of note a second male cell can be found that was not stained for EGFP (**white arrow**). **B2:** The group of fetal EGFP$^+$ cells in **A2** were male by Y chromosome FISH (**white arrow**). **B3:** Male fetal cells located in the dermis. **C:** Presence of male fetal cells in melanomas from women during pregnancy. Paraffin-embedded sections of melanoma from women bearing a male fetus or who had given birth to a male child 6 months before the surgery were analyzed by FISH. Male fetal cells were detected with two differently colored probes specific for the X (red) and Y (green) chromosomes. Photomicrographs shows male cells (**white arrow**) with X and Y chromosomes in the nucleus counterstained with DAPI (blue). **C1:** An isolated male cell in the epidermis (**white arrow**). This cell is surrounded by maternal cells (**yellow arrow**). **C2:** Group of fetal cells inside the tumor (**white arrows**). **C3:** Group of fetal cells localized in the dermis (**white arrow**). Original magnifications: ×1000 (**A1**, **C2**); ×200 (**A2**, **B3**); ×400(**A3**); ×630 (**C1**, **C3**).

optical section, triple fluorescence images were acquired. A focal series of sections was collected for each specimen. The focus step between the sections was generally 1.5 μm. Selected paired sections were then processed to produce single composite overlay images (color-merged).

Statistical Analysis

Categorical variables were compared using Fisher's exact test. For each patient, the frequency of fetal microchimeric cells per million maternal cells was determined. Student's *t*-test was used to compare the average frequencies of fetal cells in melanomas from pregnant women with the control group and others comparisons. A P value <0.05 was considered significant.

Results

Fetal Cells Selectively Invade Melanomas in Pregnant Mice

To assess the presence of fetal cells in maternal melanoma, 1 million B16-BL6 cells were injected intradermally in the right flank of 24 wild-type pregnant and nonpregnant female mice at day 5 after mating with $EGFP^+$ males. Tumors developed in all mice and were analyzed 16 days later. Among the 24 pregnant mice, 23 had at least one $EGFP^+$ fetus. Anti-EGFP immunofluorescence was performed to detect fetal cells on sections of maternal melanoma and healthy tissue (ear). $EGFP^+$ fetal cells were detected in 13 of 23 (56%) tumors and in none of the normal skin samples from pregnant mice bearing $EGFP^+$ fetus. Of note, $EGFP^+$ cells were never found in the tumors from mice that were not pregnant ($n = 15$). The fetal cells were more frequent in tumors as compared with healthy skin of the same mice (Table 1; $P = 0.000022$). Most fetal cells were found inside or surrounding the tumors. The fetal cells were either isolated or grouped in clusters (Figure 1, A1 and A2; and Supplemental Figure S1A1 available at *http://ajp.amjpathol.org*). Interestingly, in some sections, fetal cells were located in vessel walls (Figure 1A3). To increase the evidence that the observed green cells were indeed fetal, mouse Y chromosome FISH was performed on the same slides previously stained for EGFP that displayed fetal cells. Several $EGFP^+$ cells had Y chromosomes demonstrating again their fetal origin in maternal tumors (Figure 1, B1–B3). Expectedly, a few Y chromosome-positive cells could be found that did not express EGFP because these two genetic markers are independent (Figure 1B1). We did not find any Y chromosome-positive cell in nonpregnant virgin female mice. Fetal cells were also identified in five of six (83.3%) metastatic lymph nodes from pregnant mice bearing an $EGFP^+$ fetus. These fetal cells were also either isolated or grouped in clusters (Supplemental Figure S1, A and B, available at *http://ajp.amjpathol.org*).

Table 2. Clinical and Pathological Characteristics of Melanomas and Nevi in Pregnant Women Bearing Male Children

Category	Melanoma	Nevus
Number of patients	16	8
Ablation		
During pregnancy	13	8
Up to 6 months after delivery	3	0
Age at diagnosis		
Mean	28	33
Range	27 to 36	29 to 38
Breslow index (mm)		
<1.5	11	NA
≥ 1.5	4	NA
ND	1	
Mean	1.36	NA
Lymph node invasion	2	NA

Data are expressed as mean.
NA, not applicable.

Fetal Cells Are Found in Maternal Melanoma Rather than in Nevi in Pregnant Women

To further assess the validity of our findings, we examined human melanoma and benign naevus samples. Sixteen melanoma and eight nevi excised during pregnancy or in the 6 months after delivery of a male fetus were submitted to FISH. Among melanomas, 13 were excised during pregnancy (Table 2). XY^+ male cells, presumably fetal, were found in 10 of 16 melanomas as compared with one of eight nevi (62% versus 12%, $P = 0.033$; Table 1). Interestingly, the only woman who had fetal cells in a nevus during a first pregnancy developed melanoma that contained fetal cells 3 years later, during her second pregnancy. We also analyzed a control group of seven women pregnant with female fetuses and affected with polymorphic eruptions of pregnancy, an inflammatory skin disorder occurring during gestation. A single section of 21 analyzed displayed a male cell. In melanoma, fetal cells were primarily found in the dermis (10 of 10 patients), but very rarely in the epidermis (one case). These cells were isolated in the tumors (Figure 1C1) except in three patients in which groups of fetal cells were detected as detailed above in mouse melanomas (Figure 1, C2 and C3). The frequency of fetal cell invasion was 295 fetal per million maternal cells in melanoma versus 2 fetal per million maternal cells in nevi ($P = 0.034$; Figure 2).

Fetal-Derived Cells Display an Endothelial Phenotype in Melanomas Associated with Pregnancy

In mice, the phenotype of $EGFP^+$ fetal cells was assessed on the sections of mice displaying the higher number of EGFP cells. This was done using double staining with EGFP and either anti-von Willebrand factor, Lyve 1, or anti-CD45 antibodies. von Willebrand factor was observed in 56% (18 of 32 studied cells) of fetal $EGFP^+$ cells by conventional and confocal microscopy (Figure 3, A and B; and Supplemental Figure S2, B1 and S3, avail-

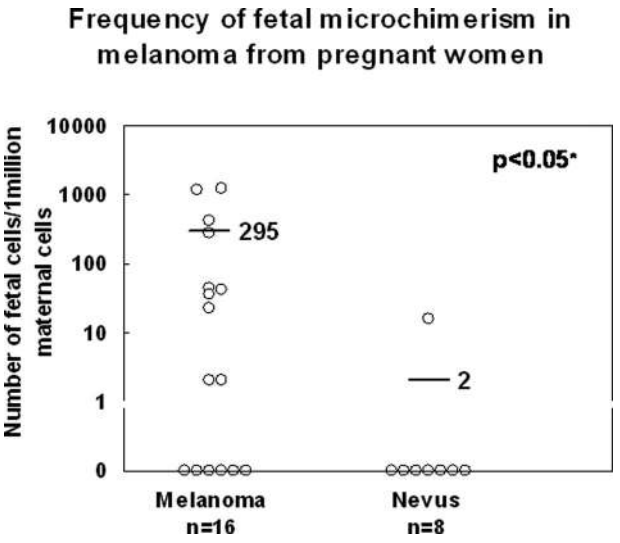

Figure 2. Frequency of fetal microchimerism in melanoma occurring during pregnancy in pregnant women. The number of male fetal cells per section is noted, and the number of maternal cells per section is calculated by multiplying the mean nuclei counted per field with the number of fields in the section. The mean number of fetal cells per million maternal cells (black lines) was significantly higher in melanoma compared with the control patients (295 versus 2 per million maternal cells; $P = 0.034$). Each dot represents a patient.*$P < 0.05$ by Student's *t*-test.

able at *http://ajp.amjpathol.org*). The pattern of staining closely resembled the one obtained in EGFP transgenic control animals, with a peripheral granular staining of endothelial cells and a more central localization of the EGFP marker (Supplemental Figure S2B2 available at *http://ajp.amjpathol.org*). To further examine the endothelial phenotype of the fetal cells, we used the lymphatic marker Lyve-1. Most fetal cells were located on a lymphatic vascular ring surrounding the tumor. After closer examination, 7 of 19 (37%) fetal cells expressed the lymphatic Lyve 1 marker (Figure 3C). A smaller proportion of fetal EGFP$^+$ cells inside the tumor expressed the CD45 antigen (20%, 3 of 15) (Figure 3D and Supplemental Figure S2C1 available at *http://ajp.amjpathol.org*). In contrast to tumors, the fetal cells in metastatic lymph nodes mainly expressed CD45 (four of eight, 50%) but not von Willebrand factor (zero of five tested) (Supplemental Figure S1, C–E, available at *http://ajp.amjpathol.org*).

We next took advantage of different possibilities to stain fetal cells by combining EGFP and Y chromosome staining in groups of fetal cells displaying endothelial markers. If EGFP-positive cells were randomly recruited to form a blood vessel, it would be expected to find 50% male and 50% female cells among them. Alternatively if the EGFP-positive cells derived from a common progenitor it would be expected that either 100% or 0% of them would be male assuming that the mendelian distribution of the pups actually occurred. In three groups of EGFP$^+$ cells stained with von Willebrand factor, a total number of 62 EGFP$^+$ cells were examined and 76% of them displayed a Y chromosome. Some of the groups were entirely male (Figure 3B) and could therefore derive from a common endothelial progenitor. However, a less probable alternative exists such that EGFP$^+$ male cells may be recruited individually to form blood vessels.

In human melanomas, FISH and immunostaining were combined to determine the phenotype of male fetal cells. Seventy-one percent of XY$^+$ fetal cells expressed the CD31 or CD34 endothelial cell markers (20 positive cells of 28 tested). Again, fetal CD31$^+$ cells were sometimes clustered, displaying the morphology of vessels (Figure 4A) localized within the tumor (Figure 4B). Two of ten fetal cells expressed the common leukocyte antigen CD45$^+$ (20%). No fetal cells expressed HMB45 or cytokeratin as a marker of melanocytes or keratinocytes, respectively.

Discussion

The relationships between melanocytic tumors and gestation have been the focus of many studies. We report here for the first time, in humans as well as in animal models with a variety of techniques, that melanomas that occur during pregnancy selectively includes fetal cells as compared with benign melanocytic nevi or healthy skin. The chimeric fetal cells are located within or around the tumor. Moreover, fetal cells appeared to have mainly adopted an endothelial phenotype, sometimes able to cluster and form vessels. This was particularly demonstrated by the use of von Willebrand factor antibody. Some of these vessels of fetal origin were lymphatics.

These findings are in accordance with previous reports of specific homing and differentiation of pregnancy-associated fetal progenitor cells in various patterns in damaged maternal organs. Indeed, fetal cells, including stem cells, enter the maternal circulation early during pregnancy and may persist for decades.[19,20] It has been shown that maternal marrow was a niche for some of the fetal transferred progenitors.[6,8] In addition, several studies in women as well as in mice have previously demonstrated the specific detection of fetal microchimeric cells in different types of tissue damage seen in various maternal tissues such as heart, liver, intestine, kidney, and even brain.[12,21–24] Of note, fetal-derived lymphoid progenitors acquired during gestation are even able to rescue immunodeficient mothers by developing into mature functional T and B lymphocytes in maternal thymus and bone marrow, respectively.[13] In maternal damaged organs, fetal cells have been shown to adopt the phenotype of the tissue itself and/or an endothelial phenotype.[10,25] Therefore, it is plausible that on tissue remodeling, fetal progenitors migrate to maternal altered tissues in a similar way as their maternal counterparts, probably through the same molecular pathways. Nevertheless, because our study did not analyze fetal cells in peripheral blood, we cannot assess whether fetal cells in maternal tumors originate from a local or distant origin. The results shown here strongly evoke the similarly to what has been shown in the above studies; fetal cells accumulate in melanoma in response to the tissue remodeling that takes place within the tumor and the surrounding tissue.

Interestingly, we recently reported that during pregnancy, fetal endothelial progenitor cells were recruited in maternal inflammatory skin and participated in the observed angiogenesis.[15] Cutaneous vessels derived from

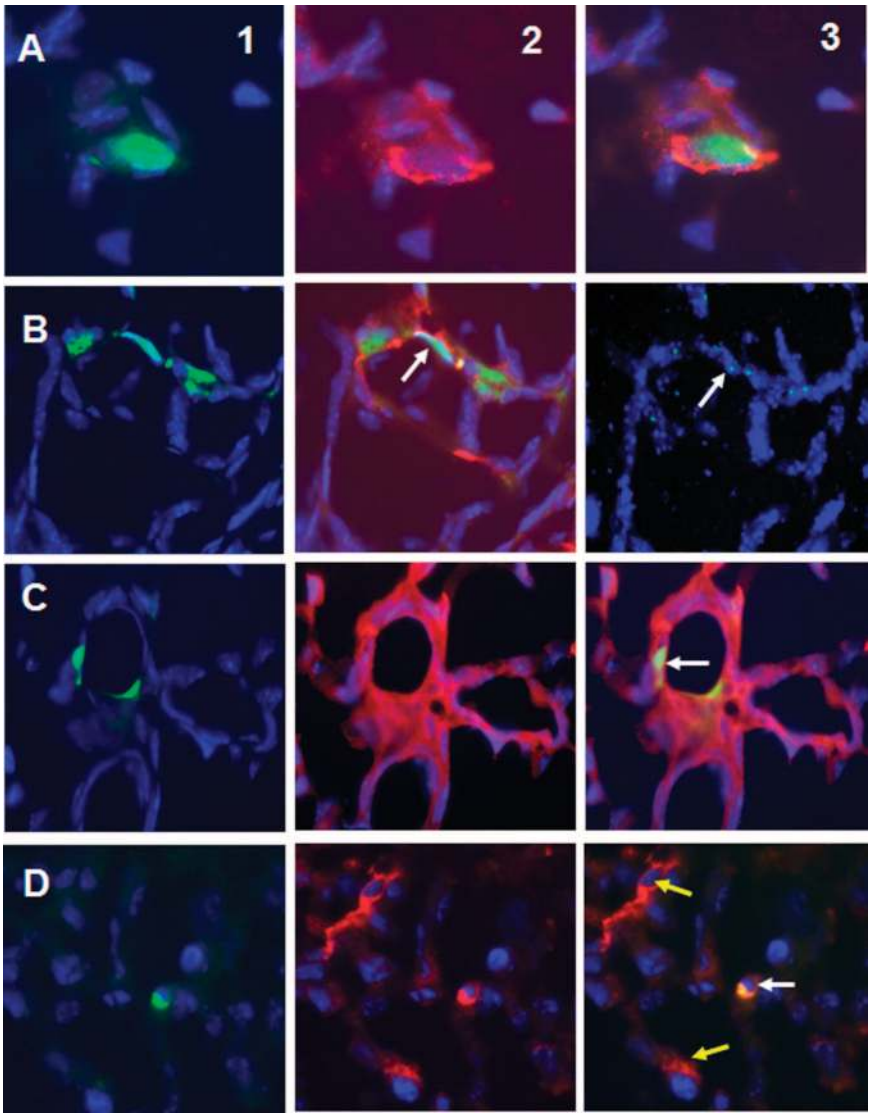

Figure 3. Fetal cells mainly express endothelial markers in mouse B16-BL6 melanoma during pregnancy. **A:** Photomicrographs show a fetal cell expressing the endothelial marker von Willebrand factor in the maternal tumor; **A1:** EGFP fluorescence (green) combined with DAPI counterstain (blue); **A2:** von Willebrand factor staining (red) combined with DAPI counterstain; **A3:** merge. **B:** Photomicrographs show fetal cells expressing the endothelial marker von Willebrand factor in blood vessels locating in periphery of the maternal tumor. **B1:** EGFP fluorescence combined with DAPI counterstain. **B2:** The fetal EGFP$^+$ cells are localized in the walls of blood vessels and express von Willebrand factor (**white arrow**). **B3:** These EGFP$^+$ cells are homogenously Y chromosome-positive (green) in nuclei with DAPI counterstain (**white arrow**) suggesting that they derive from a common progenitor. **C:** Photomicrographs show fetal cells expressing the LYVE-1 marker in lymphatic vessels of the maternal tumor. **C1:** EGFP fluorescence combined with DAPI counterstain; **C2:** LYVE-1 staining combined with DAPI counterstain; **C3:** merge. The fetal EGFP$^+$ cells are localized in the walls of lymphatic vessels and display LYVE-1 antigen (**white arrow**). **D:** Photomicrographs showing a fetal leukocyte in the maternal tumor. **D1:** EGFP fluorescence combined with DAPI counterstain; **D2:** CD45 staining combined with DAPI counterstain; **D3:** merge, an EGFP$^+$ cell in the tumor. Adjacent to this fetal cell (**white arrow**), two maternal CD45$^+$ cells can be visualized as well (**yellow arrows**). Original magnifications: ×1000 (**A3**); ×400 (**B3**, **C**, **D2**, **D3**).

fetal cells were connected to the maternal circulation, suggesting that they were functional.[15] We reproduce these findings in the present study in maternal melanoma. Indeed, fetal cells observed in maternal melanomas mainly expressed endothelial antigens. In addition to our previous study, we found similar results in human melanoma samples, emphasizing the relevance of our findings. We also showed the capacity of fetal cells to form lymphatic endothelium as well. Fetal endothelial cells usually occurred in clusters, suggesting that a single fetal progenitor may have migrated to the tumor and then proliferated to produce daughter cells. This is supported by the fact that in a single blood vessel of fetal origin, all fetal cells expressed EGFP, displaying a male karyotype by FISH as well. However, the concomitant presence of these two independent markers does not constitute a definitive proof of an endothelial progenitor. Lineage tracing experiments are needed to clearly address this point. Melanoma cells are able to induce increased blood vessel and lymphatic angiogenesis inside and at the periphery of the tumors through the secretion of several members of the vascular endothelial growth factor (VEGF) family, mainly VEGF-C and -A.[26,27] The fact that fetal-derived angiogenesis was found nearly exclusively at melanoma sites during pregnancy strongly suggests that these pathways have effectively driven fetal progenitor cells through vascular differentiation.

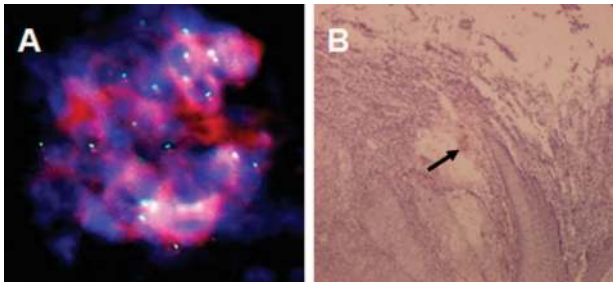

Figure 4. Presence of male fetal cells expressing the endothelial marker CD31 in melanoma of women pregnant with male children. The phenotype of fetal cells was identified by combination of FISH and immunostaining for CD31. **A:** Photomicrograph shows a group of male fetal cells expressing the vascular marker CD31 (**white arrow**). **B:** This group of male CD31$^+$ cells was located in the tumor (**black arrow**, H&E staining). Original magnifications: ×1000 (**A**); ×100 (**B**).

The prognosis of melanoma during gestation has been a subject of debate in the last 50 years. Indeed, pregnancy has been suspected to aggravate the natural course of melanoma.[28,29] Reintgen and colleagues[30] reported that the disease-free interval from 58 women with melanoma during pregnancy was shorter when compared with 2938 matched controls. Accordingly, Slingluff and colleagues[31] comparing 100 pregnant women with melanoma to 86 nonpregnant matched women found an increased incidence of lymph node metastases and a significantly shorter disease-free interval in pregnant women. More recent studies demonstrate that pregnancy does not appear an independent risk factor for metastasis at least in early stage melanoma.[32] However, the Breslow index, primary tumor thickness, representing the strongest prognostic factor, appeared higher during pregnancy in several well-conducted studies.[33,34] Because lymphangiogenesis is an important prognostic phenomenon in the development of metastases,[35,36] the presence of endothelial cells of fetal origin forming new blood vessels may suggest a deleterious role in more advanced stage melanomas. However, further specific studies are definitely needed to examine this point. Very recently, fetal cell microchimerism has been assessed in the circulation of women that previously developed a breast cancer.[37] In contrast to our findings, fetal cells were less frequent in the circulation of women with a history of breast cancer. The authors suggested that this could reflect a protective role of the fetal cells similarly to what is shown in the graft versus leukemia reaction. However, previous studies of breast carcinoma,[38] lung,[39] and cervical cancer[14]in human samples as well as skin[40] or breast carcinomas[41] in animal models have found the presence of fetal cells inside the tumor. Some of these studies have also suggested the specific homing of the fetal cells within maternal tumoral tissue as presented in our current study.[39–41] An alternate hypothesis in accordance with our findings and previous reports about fetal cell microchimerism would therefore be that fetal cells are more actively recruited to the site of tissue damage and are therefore less represented in the circulation.

In conclusion, we here report for the first time the specific homing of fetal cells in maternal melanomas during gestation. We show that fetal-derived endothelial progenitors are able to give rise to blood vessels and lymphatics, with possible consequences on the evolution of the maternal tumor. Further studies are needed to conclusively demonstrate the role of fetal microchimeric cells in maternal melanomas.

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