Fetal Cell Microchimerism and Cancer: A Nexus of Reproduction, Immunology, and Tumor Biology

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Abstract
Fetal cell microchimerism (FCM) is the persistence of fetal cells in the maternal circulation and organs following pregnancy. Proposed hypotheses about the function of fetal cells in the pathogenesis of maternal cancer include promotion of tumorigenesis, protection by providing immunosurveillance, and participation in tissue repair. To date, studies of FCM and cancer have been primarily descriptive and quantitative. More research is needed to understand the cellular phenotype of the microchimeric cells in maternal tumors and whether they have a functional role. This research will require further study using a multidisciplinary approach, incorporating knowledge of the fetomaternal relationship, tumor biology, immunology, and clinical oncology.

Introduction
Microchimerism is the presence of two genetically distinct and separately derived populations of cells in the same organ or individual (1). This state can derive from transfusion of blood products, organ transplantation, or pregnancy. Fetal cell microchimerism (FCM) refers to the persistence of fetal cells in the maternal circulation and organs for decades following pregnancy (2). It is important to note that in studies of FCM in humans, the Y chromosome is typically used as a marker of "fetal" cells, and efforts are generally made to exclude those subjects in whom microchimerism may have occurred from other sources (e.g., transfusions and transplantations). In these cases, the microchimeric cells can only be presumed to be fetal in origin. Further characterization to definitively identify these cells as fetal [e.g., human leukocyte antigen (HLA) typing] would be needed to prove their fetal origin. In animal, as opposed to human, studies, experimental conditions can be tightly controlled. These experiments show that trafficking peaks prior to parturition and declines rapidly after delivery (3, 4). The role of fetal cells in disease, including cancer, is not well understood.

Initial studies of FCM in humans tested the hypothesis that fetal cells are involved in autoimmune disease via a graft-versus-host effect, as autoimmune disorders occur more frequently in women following childbirth (5, 6). Subsequently, factors that affected the presence of FCM in humans, such as maternal-fetal histocompatibility, were identified (7, 8). To permit more extensive investigations, mouse models were developed. These showed that diverse populations of fetal cells exist in maternal organs (9), and that fetal cells can differentiate into functional lymphoid and endothelial progenitor cells (10, 11).

Recently, there has been an increase in studies of FCM and cancer in both humans and mice. Proposed hypotheses about the function of fetal cells in the pathogenesis of maternal cancer include promotion of tumorigenesis, protection by providing immunosurveillance, and participation in tissue repair. To date, the research has been primarily descriptive and quantitative. The purpose of this review is to both summarize the current status of this field and critically assess past experimental design to develop effective ways to study fetal cell involvement in maternal carcinogenesis.

Literature Review
Thus far, research involving FCM and cancer has primarily used peripheral blood and neoplastic tissue. For each published investigation, we have summarized the experimental design including study size, type of cancer examined, and methods used, as well as the authors’ primary conclusions about the putative role of fetal cells in maternal tumorigenesis (Table 1).

Peripheral Blood Studies

Breast cancer
Two studies have been published on FCM in peripheral blood and breast cancer (12, 13). PCR amplification of fetal genes in both studies showed increased numbers of chimeric cells in healthy women compared with those with breast cancer. The authors concluded that FCM protects against breast cancer by providing immune surveillance in the blood. This conclusion offers a potential explanation of why epidemiologic studies show that parous women tend to have a...
decreased incidence of breast cancer. Although these results are intriguing, it is possible that fetal cells migrate to the tumor site, leading to reduced numbers in peripheral blood.

**Solid and hematologic malignancies**

Gilmore and colleagues examined peripheral blood for FCM in healthy parous women and in parous women with both solid and hematologic malignancies using nested-PCR amplification (14). Of the women with cancer, those with solid tumors had statistically significantly lower levels of FCM than women with hematologic malignancies. These results are consistent with the hypothesis that fetal cells home to the primary site of disease, as they are present in higher numbers in the blood from hematologic malignancies compared with those with solid tumors. Unfortunately, this study did not distinguish between localized and metastatic disease. It is possible that fetal cells are only transiently present in the maternal circulation or are cleared from the circulation through migration and homing to metastatic sites. At present, the relationship between metastatic tumors and fetal cells and how this may be reflected in the maternal circulation remains unclear.

In the above studies in cases of solid tumors, fewer microchimeric cells were found in the blood of women with cancer compared with controls. The evaluation of peripheral blood, however, allows for only indirect assessment of the role of fetal cells in organ-specific disease. Future studies should incorporate analysis of peripheral blood, solid organs with primary tumors, and sites of metastatic disease to better understand the relationship between FCM in blood and tissue.

**Studies of Neoplastic Tissue**

**Cervical cancer**

One of the earliest studies of FCM and neoplasia was in cervical cancer using fluorescence in situ hybridization (FISH) with Y chromosome–specific probes (15). Eight subjects and four controls were analyzed. All of the biopsy samples that were greater than 1.5 cm² contained chimeric cells; no control tissue had evidence of male cells. These results suggested that cervical cancer might be associated with FCM; however, the status of human papilloma virus infection, a known cause of cervical cancer, was not determined in these patients.

| Table 1. Summary of published investigations on FCM and cancer |
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| Sample type | Human | Mouse | Cancer | N | Method(s) | Phenotype markers | Location and/or hypothesized role of fetal cells | Reference No. |
| Peripheral blood | √ | | Breast | 82 | PCR | None | Protection against breast cancer | 12 |
| | | | Breast | 99 | PCR | None | Protection against breast cancer | 13 |
| | | | Multiple | 200 | PCR | None | Maternal immune tolerance to paternally inherited fetal antigens | 14 |
| Neoplastic tissue | √ | | Cervical | 8 | FISH, I-FISH | CD45, CK | Associated with cervical cancer | 15 |
| | | | Thyroid | 29 | FISH | None | Associated with thyroid cancer, differentiation | 16 |
| | | | Thyroid | 63 | PCR, I-FISH | CD45, MHC class II, Tg | Maternal tissue repair, differentiation, destruction of tumor cells | 17 |
| | | | Thyroid | 106 | PCR, FISH | None | Reside in maternal niches, recruited to disease areas, differentiate | 18 |
| | | | Melanoma | 16 | I-FISH | CD31, CD34, CD45, CK | Associated with melanoma, differentiation | 19 |
| | | | Melanoma | 24 | I-FISH | CD31, CD34, CD45, CK | Associated with melanoma, differentiation | 19 |
| | | | Lung | 7 | PCR, FISH | None | Present in lung tumor, recruited from marrow, proliferate locally | 21 |
| | | | Lung | 1 | Fluorescence | None | Associated with lung tumors | 23 |
| | | | Breast | 38 | PCR | None | Protection against breast cancer | 24 |
| | | | Breast | 10 | FISH, I-FISH | CD34, CD45, CK, vimentin | Associated with breast cancer, stroma formation, differentiation | 25 |
| | | | Breast | 10 | FISH, I-FISH | CD34, CD45, CK, vimentin, vWF | Associated with breast cancer, stroma formation, differentiation | 26 |

Abbreviations: FISH, fluorescence in situ hybridization; I-FISH, combined FISH and immunohistochemistry; CK, cytokeratin; Tg, thyroglobulin; MHC, major histocompatibility complex.
**Papillary thyroid cancer**

Several studies have examined the role of FCM in papillary thyroid cancer (PTC), which predominantly affects women. One study used FISH to examine thyroid specimens in 29 women who underwent thyroidectomy, including 7 with thyroid cancer and associated thyroiditis (16). Reproductive histories included the gender of live-born children but not miscarriages and abortions. FCM was found in 5 of these 7 cases. However, it is unclear whether autoimmunity disease or the neoplastic process made a greater contribution to the trafficking of fetal cells.

Using PCR and FISH combined with immunohistochemistry (immuno-FISH), Cirello and colleagues examined 63 tumor tissue samples from women with PTC whose reproductive history included a confirmed male pregnancy before the diagnosis of cancer was made (17). Two of 19 cases in which male DNA was found had associated autoimmune thyroiditis, which may be a confounder. A follow-up study examined both blood and thyroid tissue in 57 women with PTC and 49 controls (18). Importantly, this is the only study to date that simultaneously examined trafficking in both the peripheral blood and neoplastic tissue. FCM was present in the tumors of 6 out of 57 women, but peripheral blood levels of FCM were lower in patients than healthy controls. This finding supports the hypothesis that fetal cells reside in maternal niches and can be recruited to the site of damaged tissue.

**Melanoma**

The relationship between pregnancy and melanoma is controversial (19). For example, increased hormonal binding to estrogen receptors in melanocytic lesions was first reported by Ellis and colleagues (20), whereas Lecavalier and colleagues (21) could not show a true estrogen receptor in either dysplastic nevi or malignant melanoma. This research has led to the suggestion that pregnancy does not seem to influence a woman’s risk for malignant melanoma or her prognosis (19). Nevertheless, Nguyen Huu and colleagues investigated the possible association between melanoma and fetal cells using immuno-FISH to examine both human and murine tissues for the presence of FCM (22). The human specimens were obtained from women with malignant melanoma (n = 16) or benign nevi (n = 8) if their primary tumor had been excised during (n = 21) or within 6 months (n = 3) after pregnancy. Microchimeric cells were detected in 62% of melanomas versus 12% of benign nevi and were found primarily in the dermis. Most of the chimeric cells adopted an endothelial phenotype that sometimes clustered and formed vessels. This finding is consistent with prior data that showed that in maternal organs, fetal cells adopt the phenotype of the tissue itself and/or an endothelial phenotype (23). In the murine model, males that were transgenic for the green fluorescent protein (GFP) were mated to wild-type females. One million melanoma cells were injected into female mice 5 days after mating, and tissue was examined at day e16. Fetal cells were detected in 56% of tumors and none of the normal skin samples. Fetal cells were most often found inside or surrounding tumors, as isolated cells or in clusters. Fetal cells were also identified in 83% of metastatic lymph nodes from pregnant mice (22).

**Lung cancer**

In one study using FISH and confirmed by PCR, 7 specimens were collected from women undergoing surgery for suspected lung cancer (24). Five of the six lung specimens were neoplastic: primary lung (n = 4) or metastatic from colon (n = 1). The other lung specimen was a pleural fibroma. One thymus containing benign ectopic thyroid tissue was also examined. Microchimeric cells in the diseased tissues were clustered in tumor rather than in surrounding healthy tissue. In a separate study, rib bone marrow samples from these same subjects were assessed for FCM (25). Fetal cells in maternal marrow were identified as mesenchymal stem cells on the basis of morphology, immunophenotype, and their differentiation potential. This finding suggested that marrow could act as a long-term reservoir of fetal stem cells.

In contrast, Sawicki hypothesized that fetal cells have tumorigenic potential and can act as cancer stem cells (26). She described a case in which fetal cells constituted the majority of lung tumors in a murine model. Fetal cells were easily identifiable; however, the phenotype of the cells was not explored further.

Based on these three studies, it is unclear whether fetal cells are involved in tissue repair or if they contribute to tumor growth.

**Breast cancer**

Breast cancer is more aggressive when it develops in a pregnant woman. Several studies have examined breast cancer tissues in humans and in mice. The first was a case-control study by Gadi that compared breast tissue from women with and without breast cancer and a history of having had a son (27). Cases included women undergoing mastectomy or lumpectomy for invasive ductal carcinoma or invasive lobular carcinoma with no prior chemotherapy or hormonal treatment. Controls consisted of women undergoing mammoplasty; they were significantly younger than the cases (mean 32 versus 57 years). Reproductive histories were not available. The study found that healthy breast tissue harbored more fetal microchimeric cells than diseased breast tissue.

A second study using immuno-FISH specifically examined pregnancy-associated breast cancer (28). Women carrying male fetuses and diagnosed with carcinomas during pregnancy or within 6 months after delivery were included. Controls consisted of pregnant women with benign breast tissue, such as fibroadenosis or mastosis. Nine of ten ductal carcinomas contained fetal microchimeric cells compared with zero of four benign breast lesions. Fetal cells most frequently expressed vimentin and cytokeratin, suggesting mesenchymal and epithelial origins. The fetal cells were distributed throughout the specimens but were preferentially located in areas of tumor. These results conflict with the study above (27), which could reflect differences in laboratory techniques, study design, or tumor biology.

In mice, Dubernard and colleagues examined breast tumors that developed during or following gestation (29). They used the mouse mammary tumor virus (MMTV)-H-Ras strain, which spontaneously develops breast and salivary tumors...
at about 3 months of age. Fetal cells were detected in all of the breast carcinomas and in only two of the liver controls from the same animals. High-grade tumors had significantly more fetal cells. Sixty-two percent of fetal cells expressed cytokeratin. Although this can be a very nonspecific phenotypic marker, antibodies to cytokeratin have value in determining whether these cells are epithelial cells. They are, therefore, useful in immunohistochemical studies of FCM.

**Discussion and Future Recommendations**

Overall, most investigations that have examined solid tumors convincingly show that fetal cells are preferentially present at tumor sites. The function of these cells is unknown. The hypotheses proposed in the studies cited here include promotion of tumorigenesis, provision of immunosurveillance, and participation in tissue repair.

For human studies of FCM, the type and stage of cancer, prior treatment, and time elapsed since prior treatment must be matched in controls, as tumor biology varies, invasive tumors behave differently than in situ tumors, and cytotoxic chemotherapy alters cell populations for a certain amount of time after treatment. In addition, after treatment, recurrent tumors may have a different biology from the primary tumor.

Thus far, one limitation of many human studies is that they have concentrated on women who have given birth to sons. Therefore, only half of all pregnancies can be evaluated for FCM. Animal models can overcome that limitation; they will be essential to allow large numbers of in vivo observations and to test different mechanisms of disease. Improvements in study design will also provide more robust data. Many of the studies reviewed here have been retrospective and examined FCM years after parturition, a time when fetal cell trafficking is not at its peak. It would be prudent to examine specimens at a time when trafficking is high (e.g., just prior to parturition) and at a later date when trafficking is at a presumed baseline, and to compare those time points in the clinical setting of cancer. This examination would provide information about when fetal cells can be detected in the organs of interest and how overall trafficking is altered by neoplasia. It may also provide clues about whether and how these cells home to neoplastic tissue or if they proliferate on site.

Another limitation is the methods used to both enumerate and characterize fetal cells. Most previous studies have employed PCR to quantify fetal cells. PCR has a sensitivity of 1 in 10^7 and does not provide phenotypic information (4). Studies using immuno-FISH provide a very limited interrogation of cell types identifiable by cell surface markers, as these proteins are degraded during a protease digestion step in the FISH process. One strategy to further phenotype microchimeric cells would be to use panels of antibodies that are specific for certain cell types using flow cytometry, which has a sensitivity of 1 in 10^6. Markers that are not expressed on the cell surface, such as cytokeratin, are not useful in flow cytometry experiments; thus, this can limit the range of cells that can be identified using this technique.

Lastly, a significant concern in past studies is the failure to unequivocally identify the phenotype of the fetal cells in maternal organs. The continued application of well-described techniques such as flow cytometry and immunohistochemistry using different panels of antibodies, as well as the use of techniques such as gene expression analysis, will be necessary to definitively delineate the relationship between fetal cells and cancer.

In summary, although it remains at present unclear whether fetal cells play an active role in the pathogenesis of cancer, it is important that future studies use a multidisciplinary approach that considers the fetomaternal relationship, tumor biology, immunology, and clinical oncology to incorporate knowledge of treatment and its potential side effects.

**Disclosure of Potential Conflicts of Interest**

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