Milky Spots as the Implantation Site for Malignant Cells in Peritoneal Dissemination in Mice

Akeo Hagiwara,1 Toshio Takahashi, Kiyoshi Sawai, Hiroki Taniguchi, Masataka Shimotsuma, Shinji Okano, Chouhei Sakakura, Hiroyuki Tsujimoto, Kimihiko Osaki, Sadayuki Sasaki, and Morio Shirasu

First Department of Surgery, Kyoto Prefectural University of Medicine, Kawaramachi-Hirokoji, Kamigyo-ku Kyoto 602, Japan

ABSTRACT

We examined the site-specific implantation of cancer cells in peritoneal tissues after an i.p. inoculation of 106 P388 leukemia cells. Twenty-four h after the inoculation, the number of viable cancer cells infiltrating into specific tissue sites of the peritoneum was estimated by an i.p. transfer method. A descending order of tissue implantation with cancer cells was established as omentum > gonadal fat > mesenterium > posterior abdominal wall > stomach, liver, intestine, anterior abdominal wall, and lung. A significant correlation was established between the logarithm of the number of infiltrating cancer cells and the logarithm of the number of milky spots. Next, the omentum was examined microscopically after i.p. inoculation with P388 leukemia cells labeled with bromodeoxyuridine or B-16 PC melanoma cells, which were differentiated from the other cells by an immunocytochemical method using anti-bromodeoxyuridine antibody or by the melanin of the B-16 PC melanoma cells. These cancer cells were found microscopically to be infiltrating only the milky spots, whereas none were seen at the other sites. These results suggest that cancer cells seeded i.p. specifically infiltrate the milky spots in the early stage of peritoneal metastases.

INTRODUCTION

The so-called milky spot, which is lymphoid tissue on the peritoneum, is considered to be a gate through which small particles are absorbed from the peritoneal cavity into the subperitoneum (1). The omentum possesses many milky spots (2), whereas relatively few of them are found in other areas of the peritoneum. The milky spots may contribute to the peritoneal spreading of cancer cells. Omentectomy prolongs the survival times of patients (3) and animals (4) with very early stages of peritoneal carcinomatosis. Furthermore, from clinical observations it is known that cancers disseminated i.p. are frequently found in the greater omentum and the pelvic floor, where large numbers of milky spots distribute, than in other peritoneal locations. These facts lead us to propose that cancer cells seeded in the peritoneal cavity could infiltrate through the milky spots in the early stages of peritoneal dissemination.

However, because of the difficulties in estimating the numbers of viable cancer cells infiltrating the peritoneal and subperitoneal tissues, no human or animal experiments have established a quantitative procedure for measuring the infiltration of these cells into the peritoneum at specific locations. This paper addresses this methodological problem by using a new experimental approach to determine the number of cancer cells infiltrating the peritoneal surface and subperitoneal tissues sampled from various intraperitoneal locations. With a reliable quantitative technique in hand we were able to establish a significant correlation between the number of milky spots at specific peritoneal locations and the number of infiltrating cancer cells. Finally, we were able to demonstrate microscopically cancer cells infiltrating specifically at the milky spots.

MATERIALS AND METHODS

Animals and Cancer Cell Line. Five-week-old male CDF1 mice (Shimizu Laboratory Animal Center, Kyoto, Japan) were maintained under standard conditions (specific pathogen-free; 22°C; 60% relative humidity; 12-h day-night cycle).

P388 leukemia cells were maintained through i.p. inoculation in DBA2Cr mice (Shimizu Laboratory Animal Center). The ascites containing P388 leukemia cells was taken from the carrier mouse and suspended in saline at a concentration of 107 P388 leukemia cells/ml. The viability of the tumor cells, as determined by the trypan blue exclusion test, was greater than 95%. The tumor cell suspension was used within 4 h of its preparation.

Calibration Line of P388 Leukemia Cell Number. An experiment was carried out to obtain a calibration curve from which the number of viable P388 leukemia cells inoculated i.p. could be calculated. One hundred and twenty mice were divided into 6 groups of 20 mice each. A P388 leukemia cell suspension, prepared as described above, was diluted with saline into 6 different concentrations of tumor cell suspensions containing either 107, 106, 105, 104, 103, or 102 cells/ml. Each group of 20 mice received an i.p. injection of 1 ml/mouse of each concentration of P388 leukemia cell suspension. The number of survivors was checked daily for 60 days, and the average survival time was plotted against the number of P388 leukemia cells inoculated i.p. on a semilogarithmic abscissa.

Cancer Cell Number Assay in Peritoneal Dissemination. Twenty mice received an i.p. inoculation of 106 P388 leukemia cells/mouse. Twenty-four h after inoculation, the mice were sacrificed. Nine different tissues, i.e., the whole of the greater omentum (8 mg/mouse on average), and 25 mg/mouse of tissues sampled from the parietal peritoneum and subperitoneum of the posterior abdominal wall around the kidneys and the paraoartic tissues, the surface of the liver edge, both upper edges of the gonadal fat, the mesenterium of the small intestine, the anterior wall surface of the stomach, the wall surface of the small intestine, the anterior abdominal wall including the parietal peritoneum, and the lung were sampled from each mouse. To wash the P388 leukemia cells from the tissue surfaces, the tissue samples were rinsed twice in 500 ml of saline. Each tissue sample was individually minced with scissors into tissue fraction suspension in 1 ml of saline under aseptic conditions. Each tissue fraction suspension was individually transferred i.p. to a normal (non-cancer-bearing) mouse. A total of 180 of these assay mice were used; the cell suspension for each of the above-mentioned 9 different sampled tissues was given to 20 mice. Following the i.p. injection of the tissue fraction suspensions, the assay mice were observed daily for 60 days, and the days of death were recorded. From the previously obtained calibration curve, the number of cancer cells infiltrating each tissue was estimated from the survival time of the corresponding assay mouse. The survival time was compared by the general Wilcoxon's test between the assay mice groups to see the statistical difference in the number of viable P388 leukemia cells transferred from the various types of tissues to the assay mice.

Milky Spot Number Assay. Peritoneal milky spots, which were aggregates of macrophages and lymphocytes on the peritoneal surface, were detected with two different methods. As macrophages take particles such as activated carbon into their cytoplasm, macrophages in milky spots are visualized as blackened cells after i.p. injection of activated carbon (5). The second method is a nonspecific esterase stain, which colors monocytes (macrophages) and T-lymphocytes located in the milky spots dark red (6).

Twenty mice received a 1-ml i.p. injection of activated carbon suspension (activated carbon CH-40, 10 mg/ml in saline). Four weeks later, when all of the intrapertoneal carbon particles had been taken into the cytoplasm of the macrophages in the milky spots, the mice were sacrificed, and 8 of 9 tissues mentioned above (the lung was excluded) were prepared for the nonspecific

Received 8/12/92; accepted 11/18/92.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked advertisement in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

1 Supported in part by a Grant-in-Aid for Cancer Research from the Ministry of Health and Welfare, and from the Ministry of Education, Science and Culture, Japan.

2 To whom requests for reprints should be addressed.

687
estrous cycle according to the method of Horwitz et al. (6). The specimens were set and compressed between two glass slides into thin specimens for microscopic examination under a stereomicroscope at \( \times 40 \) magnification. The slides which were colored by both the nonspecific esterase staining and the activated carbon staining were regarded as milky spots. For each specimen the number of milky spots was counted in 5 randomly selected visual fields that were 5.1 mm in diameter. The number of milky spots for one specimen was calculated as:

\[
\text{Mean of the milky spot numbers in the 5 visual fields} = \frac{\text{One visual field}}{5}
\]

In this assay, when an average of one milky spot was found in 98 visual fields, the milky spot number was considered to be zero per visual field, with a probability of greater than 95%. in spite of the fact that the actual milky spot number is 1/98 visual fields = 0.0102/visual field. Therefore, the assay limit was 0.01 milky spots/visual field.

The difference in the milky spot numbers between the various intraperitoneal locations was compared by analysis of variance.

**Correlation between Milky Spot Number and Infiltrating Cancer Cell Number.** The variable \( X \), which represents the logarithm of the mean implanted cancer cell number in each specific tissue, was plotted against the variable \( Y \), which represents the logarithm of the milky spot number for the corresponding specific tissue. This log-log plot permits the determination of the multiplicative value of the variable \( X \) when the mean of the milky spot number for a tissue is greater than the assay limit of 0.01/visual field. When the milky spot number distribution on the tissue is less than the assay limit, the milky spot number is taken as zero, for which the logarithm cannot be calculated. Therefore, these data were discarded. The equation:

\[
Y = BX + A
\]

equation expressing the correlation between \( X \) and \( Y \) was obtained statistically by single regression analysis.

By using the above-determined multiplier value \( B \), multiple regression analysis was used to fit the data in the 8 intraperitoneal locations to the equation:

\[
Y = CX^2 + A
\]

where \( Y \) represents the milky spot number and \( X \) represents the infiltrating cancer cell number.

**Immunocytological Staining of P388 Leukemia Cell Infiltration.** To determine the sites where the P388 leukemia cells infiltrated the peritoneum, an immunocytological staining method (7, 8) with monoclonal anti-BrdUrd \(^3\) antibody (9) (Becton Dickinson Immunocytometry System Co., Ltd., San Jose, CA). Finally, the specimens were counterstained with methylene green, dehydrated, and mounted for stereomicroscopic observations.

**Histological Observation of B-16 PC Melanoma Cell Infiltration.** The mouse B-16 PC melanoma cell line, which has been recently established from the standard B-16 melanoma through serial i.p. inoculation (24 times) in our laboratory and maintained by cell culture in vitro, easily induces peritoneal dissemination by i.p. inoculation. B-16 PC melanoma cells at \( 10^7 \) cells/mouse were inoculated i.p. into 5 mice (5-week-old male C57/BL6; Shimizu Laboratory Animal Center). Four days after inoculation, the mice were sacrificed. The 8 kinds of different i.p. tissues which were examined in the experiments on the infiltration of P388 leukemia were sampled and prepared for histological specimens, which were stained by methylene green in order to contrast the black melanoma cells with the nuclei of the aggregating lymphocytes and monocytes in the milky spots.

**RESULTS**

**Calibration Curve for Cancer Cell Number.** The relationship between the survival time and the logarithm of the cancer cell number was linear. The calibration curve from which the number of viable P388 leukemia cells was estimated was defined by the equation:

\[
T = -2.5 \log N + 22.5
\]

where \( T \) is the survival time of the mice in days and \( N \) is the number of P388 leukemia cells/mouse.

**Infiltrating Cancer Cell Number in Tissues.** The number of viable P388 leukemia cells/25 mg of tissue sample infiltrating 9 specific locations is summarized in Table 1. By far the largest number of cells was found in the omentum, with more than 1 million cells/25 mg of tissue sample, whereas all other tissues contained substantially fewer cells. Gonadal fat contained approximately 11,000 cells, the mesentery almost 2,500 cells, and the posterior abdominal wall 230 cells/25 mg of tissue sample. All of the other 5 tissues had fewer than 10 cells/25 mg of tissue sample.

The statistical significance of the number of infiltrating cancer cells for the different tissues was examined by comparing the survival curves of the various assay mice groups (Fig. 1). The survival curve of the omentum group was significantly different from that of all other tissue groups \((P < 0.001)\), with calculated \( Z \) values of 4.65, 5.03, 5.33, 5.63, 5.71, 5.71, 5.75, and 5.80 for the gonadal fat group, the mesenterium group, the posterior abdominal wall group, the small intestinal wall group, the gastric wall group, the anterior abdominal wall group, the liver group, and the lung group, respectively. The survival curve of the gonadal fat group was not significantly different from that of the mesenterium group \((P > 0.05)\), but was significantly different from the survival curves of the posterior abdominal wall group \((P < 0.01); Z = 2.96)\), the small intestinal wall group, the gastric wall group, the anterior abdominal wall group, the liver group, and the lung group \((P < 0.001); Z = 4.10, 4.27, 4.27, 4.30, 4.45)\), respectively. The survival curve of the posterior abdominal wall group was significantly different, as compared with the small intestinal wall group, the gastric wall group, the anterior abdominal wall group, the liver group, and the lung group \((P < 0.001)\) in the mesentery group, the posterior abdominal wall group, the small intestinal wall group, the gastric wall group, the anterior abdominal wall group, the liver group, and the lung group \((P < 0.001)\), respectively. The survival curve of the posterior abdominal wall group was significantly different, as compared with the small intestinal wall group, the gastric wall group, the anterior abdominal wall group, the liver group, and the lung group \((P < 0.001); Z = 3.56, 3.77, 3.77, 3.78, and 4.01, respectively\). The survival curve of the posterior abdominal wall group was significantly different, as compared with the small intestinal wall group, the gastric wall group, the anterior abdominal wall group, the liver group, and the lung group \((P < 0.001); Z = 4.10, 4.27, 4.27, 4.30, 4.45)\), respectively. The survival curves of the other 5 groups were not significantly different \((P > 0.05); Z < 1.43)\). There was no dead mouse in the lung group.

**Milky Spot Number.** A milky spot on the peritoneal surface, detected using activated carbon stain and nonspecific esterase stain, is shown in Fig. 2. Macrophages containing black particles of activated carbon and T-lymphocytes, the granules of which are turned dark red by nonspecific esterase stain, were aggregated. The number of milky spots for the various tissues is shown in Table 2. Mean milky
CANCER INFILTRATION IN MILKY SPOTS

Table 1 Tissue locations of P388 leukemia cells

<table>
<thead>
<tr>
<th>Tissue sites</th>
<th>Infiltrating cancer cell number (cells/assay mouse = cells/25 mg of tissue sample)*</th>
<th>Statistical difference*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Omentum</td>
<td>(1.32 \times 10^6) ([4.23 \times 10^5])</td>
<td>(P &lt; 0.001)</td>
</tr>
<tr>
<td>Gonadal fat</td>
<td>(1.11 \times 10^6)</td>
<td>NS</td>
</tr>
<tr>
<td>Mesenterium</td>
<td>(2.46 \times 10^5)</td>
<td>(P &lt; 0.01)</td>
</tr>
<tr>
<td>Posterior abdominal wall</td>
<td>(2.30 \times 10^2)</td>
<td>(P &lt; 0.05)</td>
</tr>
<tr>
<td>Liver</td>
<td>6.25</td>
<td>(P &lt; 0.001)</td>
</tr>
<tr>
<td>Gastric wall</td>
<td>1.83</td>
<td>NS</td>
</tr>
<tr>
<td>Anterior abdominal wall</td>
<td>1.83</td>
<td></td>
</tr>
<tr>
<td>Small intestinal wall</td>
<td>0.75</td>
<td></td>
</tr>
<tr>
<td>Lung</td>
<td>0</td>
<td></td>
</tr>
</tbody>
</table>

* Cancer cell number was estimated from the survival times of the assay mice, into which 25 mg/mouse of tissue containing cancer cells was transferred.
* Statistical significance was estimated from the survival curve of the assay mice using the generalized Wilcoxon's test.
* The number of cancer cells in 8 mg of omental tissue transferred to an assay mouse.
* NS, not significant; arrows, statistical significance between locations.

Spot numbers per visual field were 21.8 (95% CI, 20.1–23.5), 1.60 (95% CI, 1.37–1.83), 0.610 (95% CI, 0.448–0.772), 0.150 (95% CI, 0.092–0.208), and 0 (95% CI, 0–0) for the omentum, gonadal fat, mesenterium, posterior abdominal wall, and for the other 4 intra-abdominal locations, respectively. These numbers of milky spots were all significantly different from one another \((P < 0.005; F = 29.3–554)\). The liver, the gastric wall, the anterior abdominal wall, and the small intestinal wall had significantly fewer numbers of milky spots than were detectable with the assay (0.01 spots/visual field), and thus these 4 tissues were considered to have no spots.

Correlation between Infiltrating Cancer Cell Number and Milky Spot Number. Since in 4 of the intraperitoneal locations (i.e., the omentum, the gonadal fat, the mesenterium, and the posterior abdominal wall) the mean milky spot numbers were significantly greater than assay limit (0.01/visual field), these tissues were used to determine the relationship between milky spot number and the number of infiltrating cancer cells. These variables were related by the equation:

\[ Y = BX + A \]

where the constant \(B = 0.574\) (95% CI, 0.522–0.627) and represents the multiplier value of the mean number of infiltrating P388 leukemia cells/25 mg of tissue sample; the constant \(A = -4.97\) (95% CI, -5.48 to -4.47); the variable \(X = \ln(\text{mean number of infiltrating P388 cells/25 mg of organ tissue})\); and the variable \(Y = \ln(\text{mean number of milky spots/visual field})\) (degrees of freedom = 3; correlation index = 0.9995; \(F = 2111\); \(P < 0.0005\)). Thus, milky spot number and (cancer cell number)\(^{0.574}\) correlated linearly with a high degree of significance.

A regression analysis of the number of infiltrating cancer cells and the number of milky spots for the 8 intraperitoneal tissues gave the equation:

\[ Y = CX^{0.574} + 0.0237 \]

where the constant \(C = 0.00663 \pm 0.00002\) (mean \(\pm\) SE); \(Y\) is the mean milky spot number per visual field \((Y = \ln Y)\); and \(X\) is the mean viable cancer cell number/25 mg of tissue sample \((X = \ln X)\) \((P < 0.0005; F = 68382;\) coefficient of determination = 0.9999; multiple correlation of coefficient = 0.9999; \(T = 261\)). \(C = 0.00663\) was very similar to:

\[ e^A = e^{-4.97} = 0.007 \]

which was obtained in a correlation test. The other constant value of 0.0237 was very small and near the limit of the milky spot number assay (0.01).

Microscopic Study of Cancer Infiltration with P388 Leukemia and B-16 PC Melanoma. An immunocytological staining study showed that BrdUrd-labeled P388 leukemia cells infiltrated the milky spots but no other sites (non-milky spot sites) in the peritoneum (Fig. 3). In the control specimens, no such cells were seen.

Microscopic examination using B-16 PC melanoma also showed that B-16 PC melanoma cells were found infiltrating selectively into the milky spots, whereas no B-16 PC melanoma cells were seen in the other sites of the peritoneum (Fig. 4).

DISCUSSION

We used P388 leukemia cells as an experimental tumor. One of the reasons for this is that a linear correlation between the survival time of the mice and the logarithm of the number of P388 leukemia cells...
CANCER INFILTRATION IN MILKY SPOTS

Fig. 2. Milky spot detected by both the activated carbon method and the nonspecific esterase staining method. Milky spots are detected as the aggregations of macrophages, seen as blackened cells by the activated carbon and T-lymphocytes turned dark red by nonspecific esterase staining. Stereomicroscopic view. X 20.

<table>
<thead>
<tr>
<th>Peritoneal locations</th>
<th>Milky spot number</th>
<th>(95% CI)</th>
<th>Statistical differences</th>
</tr>
</thead>
<tbody>
<tr>
<td>Omentum</td>
<td>21.8</td>
<td>(20.1–23.5)</td>
<td>( P &lt; 0.005 )</td>
</tr>
<tr>
<td>Gonadal fat</td>
<td>1.60</td>
<td>(1.37–1.83)</td>
<td>( P &lt; 0.005 )</td>
</tr>
<tr>
<td>Mesenterium</td>
<td>0.61</td>
<td>(0.45–0.77)</td>
<td>( P &lt; 0.005 )</td>
</tr>
<tr>
<td>Posterior abdominal wall</td>
<td>0.15</td>
<td>(0.09–0.21)</td>
<td>( P &lt; 0.005 )</td>
</tr>
<tr>
<td>Four others*</td>
<td>0</td>
<td>(0–0.0)</td>
<td></td>
</tr>
</tbody>
</table>

* Arrows, statistical significance between tissues.

Inoculated into the peritoneum (10) suggests its usefulness as an assay for the number of cancer cells that transferred with specific tissue fractions obtained from mice previously inoculated with the P388 leukemia cells. We have applied this methodology to determine the numbers of P388 leukemia cells infiltrating various intraperitoneal locations following an inoculation of the cancer cells into the peritoneal cavity.

The second reason for using this cell line is that P388 leukemia has been used in an experimental model of cancer metastasis to the regional lymph nodes (10). When P388 leukemia cells are inoculated s.c. in that experimental model, the cells metastasize not to the lymphatic tissues located systemically but only to the regional lymph node, just like lymph node metastases of carcinomas. In our preliminary experiment using the detection of viable P388 leukemia cells with the i.p. transfer method, no viable P388 leukemia cells were found in the mesenteric lymph nodes or the retroperitoneal nodes which are located in the subperitoneum, or in other nodes such as the axillary lymph nodes, whereas many viable P388 leukemia cells were detected in peritoneal locations, where many milky spots were distributed, 24 h after i.p. inoculation of \( 10^6 \) P388 leukemia cells. Moreover, our experiment using B-16 PC melanoma showed that cells inoculated i.p. infiltrated selectively at the milky spots, as did P388 leukemia cells. These findings suggest that intraperitoneal P388 leukemia cells behave in the same manner as carcinomas in peritoneal metastases.

Another preliminary experiment showed that 2 days after the inoculation of \( 10^5 \) P388 leukemia cells, viable cancer cells were detected in the lung tissues in 2 of 10 mice. This finding suggests that 2 days after the inoculation the cancer cells can be implanted into intraperitoneal tissues through hematogenous metastases as well as through peritoneal seeding. Since we found, in the present study, that hematogenous metastasis of P388 leukemia cells to the lungs did not occur at 24 h after inoculation, we used this time to estimate the frequency at which the injected cells infiltrate into various tissues in the peritoneal cavity.

No quantitative study on site specificity of peritoneal carcinomatosis has been performed to date. Wheatley et al. (11), however, have examined microscopically the adhesion of peritoneally disseminating cells at 11 different sites on the peritoneal surface. The mesentery had adherent cancer cells on 3 days after intraperitoneal inoculation, whereas on the surface of other organs the cancer cells did not adhere until much later. Neither Wheatley et al. (11) nor other investigators (12, 13) who studied cancer infiltration in the peritoneal surface described cancer cells infiltrating through the milky spots.
In the present study we found that milky spots exhibit a very location-specific distribution in the peritoneum and that the location-specific infiltration of P388 leukemia cells correlated significantly with the distribution of the milky spots: the relationship between these two variables is precisely described by the exponential formula:

\[ Y = 0.00663X^{0.574} + 0.0237 \]

where \( Y \) is the milky spot number and \( X \) is the P388 leukemia cell number. The constant value of 0.0237, which is a little larger than the detection limit of 0.01 milky spots/visual field, suggests that in 100 visual fields only two milky spots will have no cancer cell infiltration. This is a very small proportion when compared to the 2180 milky spots in 100 visual fields for the omentum. Therefore, after an i.p. injection of cancer cells, very few milky spots will not be infiltrated by the cells, and those milky spots without cancer infiltration will hardly modify the extent of cancer cell infiltration into the peritoneal tissues.

As for peritoneal tissues without milky spots, only 6.25 or fewer cancer cells infiltrated per 25 mg of tissue weight. Thus, although some cancer cells can infiltrate through nonmilky spots, the number of cancer cells infiltrating by this route have a smaller effect on the overall infiltration rate of cancer cells than do milky spots which have no cancer cells.

Our histological observations using BrdUrd-labeled P388 leukemia cells and B-16 PC melanoma showed that these malignant cells
infiltrated the milky spots after i.p. inoculation, but these cells did not infiltrate other sites on the peritoneum.

In mice, the peritoneum of male mice extends from the peritoneal cavity to the inner side of the scrotum as two serosal sacs. The gonadal fat is located at the opening of the sac, and the gonadal organs (the testes) are suspended from the peritoneal cavity in the serosal sacs of the scrotum. In human beings, however, the neck of the serosal sac which is formed by the extending peritoneum adheres and, together with the tissues corresponding to the gonadal fat in rodents, makes a part of the pelvic floor during the growth of the fetus. Thus, the gonadal fat in male mice corresponds to a part of the pelvic floor in human beings.

These results suggest that cancer cells seeded i.p. infiltrate specifically at the milky spots during the initial stage of peritoneal metastases. Consequently, peritoneal dissemination of cancer cells occurs predominantly in the omentum, mesenterium, and human pelvic floor, corresponding to the gonadal fat in rodents, where many milky spots are distributed.

REFERENCES

Milky Spots as the Implantation Site for Malignant Cells in Peritoneal Dissemination in Mice

Akeo Hagiwara, Toshio Takahashi, Kiyoshi Sawai, et al.


Updated version
Access the most recent version of this article at:
http://cancerres.aacrjournals.org/content/53/3/687

E-mail alerts
Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions
To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions
To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.