Bottom-up Histogenesis of Colorectal Adenomas: Origin in the Monocryptal Adenoma and Initial Expansion by Cryp Fission


Histopathology Unit, London Research Institute, Cancer Research UK, London WC2A 3PX, United Kingdom [S. L. P., R. P., R. J., R. A. G., N. M., G. E., N. A. W.]; Department of Histopathology, Barts and the London, Queen Mary’s School of Medicine and Dentistry, London E1 2AD, United Kingdom [S. L. P., N. A. W.]; Department of Medicine, Queen Mary Hospital, Hong Kong [W.-M. W., A. O.-O. C.]; Department of Histopathology, Royal Free and University College Medical School, London NW3 2PF, United Kingdom [M. N.]; Cancer and Immunogenetics Laboratory, Cancer Research UK, Weatherall Institute of Molecular Medicine, Oxford OX3 9DS, United Kingdom [W. F. B.]; Molecular and Population Genetics Laboratory, London Research Institute, Cancer Research UK, London WC2A 3PX, United Kingdom [I. P. T.]; and Colorectal Unit, Cancer Research UK, St. Mark’s Hospital, Harrow, Middlesex HA1 3UJ, United Kingdom [I. P. T.]

ABSTRACT

The adenoma:carcinoma sequence is well established. Understanding the molecular pathology of the adenoma is therefore important. There is great controversy within the field. The Vogelstein group champions the “top-down” theory (colorectal adenomas arise and grow across the mucosal surface and down into the crypts), whereas other studies, including our own, propose “bottom-up” spread. Serial sections of 40 small (<3 mm) sporadic colorectal adenomas were stained with H&E, MB-1, and for β-catenin. 10 early adenomas were Feulgen-stained and microdissected. We also examined the flat mucosa of three patients who had undergone colectomies for familial adenomatous polyposis (FAP) and specimens from a XO/XY individual with FAP, the latter using in situ hybridization for the Y chromosome. In the earliest sporadic adenomas, there were crypts entirely filled with adenomatous epithelium, which showed proliferative activity and nuclear and luminal surface of colonic crypts (3); assessment of LOH for APC and nucleotide sequence analysis of the mutation cluster region of the APC gene was applied to microdissected, well-oriented histological sections of these adenomas. Half the sample showed LOH in the upper portion of the crypts, and most of these had a truncating APC mutation. Those cases without LOH showed a truncating mutation, again confined to the dysplastic epithelium at the crypt apex. Moreover, only these superficial cells showed intense proliferative activity, with nuclear localization of β-catenin, supporting the presence of an APC mutation in these apical, dysplastic cells. Several previous morphological studies have drawn attention to the same appearances, including those in FAP (5). Such a “top-down morphogenesis” has profound implications for concepts of stem cell biology in the gut. Most evidence indicates that crypt stem cells are found at the origin of the cell flux, near the crypt base (6). Their repertoire includes all crypt cell lineages, metaplastic and reparative cell lineages, the genesis of new crypts, and, as is widely believed, gastrointestinal tumors (4, 6). These proposals by Shih et al. (3) either reestablish the stem cell compartment in the intracryptal zone or make the intracryptal zone a locus where stem cells, having acquired a second hit, clonally expand. An alternative hypothesis involves the recognition of the earliest lesion, the unicryptal or mononuclear adenoma, where the dysplastic epithelium occupies an entire single crypt (7). These lesions are very common in FAP, and although they are rare in non-FAP patients, they have certainly been described (8). Here a stem cell apparently acquires a second hit and expands (either stochastically or more probably because of a selective advantage, to colonize the entire crypt). Such mononuclear lesions thus should be clonal (9). Similar crypt-restricted expansion of mutated stem cells has been well documented in mice after ethyl nitrosurea (ENU) treatment (10) and also in humans heterogeneous for the OAT gene, where, after LOH, initially half and then the whole crypt is colonized by the progeny of the mutant stem cell (11). Interestingly, OAT+/OAT− individuals with FAP show increased rates of stem cell mutation with clustering of mutated crypts (12). In this scenario, in sharp contrast, the mutated clone further expands, not by lateral migration but by crypt fission, where the crypt divides, usually symmetrically at the base, or by budding (Fig. 1A). In several studies, fission of adenomatous crypts is regarded as the main mode of adenoma progression, certainly in FAP, where such events are readily evaluated (13, 14), but also in sporadic adenomas (15). In fact, the nonadenomatous mucosa in FAP, with only one APC mutation, shows a large increase in the incidence of crypts in fission (13). Aberrant crypt foci, lesions that are putative precursors of adenomas,
which can show k-RAS and APC mutations (16), grow by crypt fission (17, 18), as do hyperplastic polyps (19). But this concept does not exclude the possibility that the clone later expands by lateral migration and downwards spread into adjacent crypts, with the initial lesion being the monocryptal adenoma; however, this model of morphogenesis is conceptually very different from that proposed from Shih et al. (3)

Here we analyze very early sporadic adenomas and the earliest lesions we could identify in cases of FAP, and we conclude that (a) the earliest lesion in the development of both sporadic and FAP adenomas is likely to be the monocryptal adenoma, (b) early clonal expansion is through crypt fission, and (c) spread via the surface is likely to be a phenomenon confined to the later stages of evolution of colorectal adenomas.

MATERIALS AND METHODS

Early Sporadic Adenomas. Forty tiny (≤3 mm in diameter) tubular adenomas were available from 26 patients and removed at colonoscopy. The procedures were performed on routine endoscopy lists for a variety of clinical reasons (e.g., anemia, altered bowel habit). Three other tubular adenomas of larger size were also studied (up to 1 cm in diameter). The tissues were embedded in Paraplast, and serial sections were cut at a thickness of 4 μm. They were then stained with hematoxylin, for β-catenin (Transduction Labo-

Fig. 1. A, scheme for crypt fission and examples of a microdissected single crypt and a bifurcating crypt. i–iii, the process of mutational crypt clearance after injection of ethynitrosourea. i, hemi-cryptal loss of glucose-6-phosphate dehydrogenase (G6PD) staining; ii, monoclonal conversion of the whole crypt to the mutated phenotype; iii, expansion of the mutant clone to a patch of eight crypts, by the process of crypt fission (from Ref. 10, with permission). B–D show β-catenin in the nuclei of adenomatous crypts. E shows that nuclear β-catenin extends to the bottom of crypts in early adenomas, including the very bases of the crypts. F and G, β-catenin in nuclei of budding crypts. H, adenomatous crypts from a larger adenoma stained for β-catenin showing invasion of adjacent crypt territories in a top-down fashion. I, widespread distribution of MIB-1 staining in the crypts of early adenomas. J, another area showing surface continuity between crypts with nuclear β-catenin staining. K, the junction of an adenomatous crypt in an early adenoma with the surface epithelium, showing a sharp junction where the nuclear accumulation of β-catenin gives way to membrane staining in the normal surface cells. L, the next serial section (to K), demonstrating the sharp junction between the nuclear staining in the adenomatous cells and the membrane staining in the normal surface cells.

Downloaded from cancerres.aacrjournals.org on November 9, 2017. © 2003 American Association for Cancer Research.
ory, Becton Dickinson, Middlesex, United Kingdom), and for Ki67 using the MIB-1 antibody (Dako Ltd., Cambridgeshire, United Kingdom; Ref. 20). Immunohistochemistry was performed using the streptavidin-biotin immuno-
peroxidase method. Sections were de waxed, rehydrated, and incubated with
0.3% hydrogen peroxide (H$_2$O$_2$) for 30 min to block for endogenous peroxi-
dase activity. Antigen retrieval was performed by microwave exposure in 0.01
M citrate buffer (pH 6.0) at 750 W. After microwaving for 10 min, the sections
were incubated in anti-β-catenin (mouse monoclonal antibody) for 35 min at
a concentration of 1:100. They were then washed in PBS before incubation
with biotinylated rabbit antimouse antibody (1:300; Dako) for 30 min, washed
in PBS, and then incubated with streptavidin-peroxidase conjugate (1:500;
Dako) for an additional 30 min. The sections were developed with 3,3'-
diaminobenzidine-tetrahydrochloride (Sigma-Aldrich, Dorset, United King-
dom) and 0.1% H$_2$O$_2$ solution for 2 min and counterstained with hematoxylin.
MIB-1 was used at a concentration of 1:100, again after microwaving for
antigen retrieval (20 min). The secondary and tertiary layers were as described
with β-catenin. For negative controls, duplicate sections were used in which
the primary antibodies were omitted and replaced with PBS.

Microdissected Material. Small (<10 mm) fresh adenomas and normal
colonic mucosa were obtained by endoscopic means during colonoscopy after
informed written consent. The clinical indications were similar to those of
the early adenoma group. Part of the colonic polyps were fixed in formalin and
sent for routine histological examination. Ten adenomas (six tubular adenomas
and four tubulovillous adenomas) were available for study. Noninvolved
colonic mucosa 10–15 cm away from the lesions was also studied. These
adenomas have been used in a previous study by Wong et al. (21). In addition,
five samples of normal colonic mucosa from patients with normal colonolo-
scopic findings were used as controls.

Individual crypts were microdissected using the method described by Good-
lad et al. (22). The tissue was taken from its storage in 70% ethanol, hydrated
in 50% ethanol for 10 min followed by 25% ethanol for 10 min, and
hydrolyzed in 1 M HCl at 60°C for 10 min. It was then stained by Feulgen
reaction for at least 45 min. The tissue was transferred onto a slide with 45%
acetic acid and gently teased apart under a dissecting microscope (at ×25
magnification). A coverslip was then placed over the wet tissue and tapped
until the crypts began to separate.

FAP Adenomas. Paraffin-embedded tissues from three cases of FAP who
had undergone total prophy lactic colectomy were studied. Fifteen blocks
(Swiss rolls to identify both the flat mucosa and the adenomas) were embeded
from each patient, with approximately 20 serial sections taken from each block
at several levels. Serial sections were cut at 4 μm and stained with H&E.

In Situ Hybridization. Tissue was also available from the XO/XY patient
we have described previously (9). More sections were cut at 4 μm and underwent in situ hybridization for the Y chromosome as described in the same
article.

RESULTS

Early Sporadic Adenomas: Histological Studies. These tubular
adenomas consisted of straight or coiled tubules, filled with cells
showing varying degrees of atypia. All adenomas examined showed
evidence of budding (Fig. 1, F and G), and careful examination
showed that all adenomas also show multiple buds in some crypts.
In accord with the literature, 40% of the adenomas showed nuclear
accumulation of β-catenin (Ref. 23; Fig. 1, B–E), suggesting that loss
of function in one of the genes in the Wnt pathway, most likely APC,
has occurred with subsequent translocation of β-catenin to the
nucleus. Closer examination of serial sections shows that the β-catenin
nuclear staining extends to the bottom of the adenomatous crypts (Fig.
1, B–E) and is present in crypts in the process of crypt fission (Fig. 1,
F and G); β-catenin expression is particularly marked in the nuclei of
buds. Moreover, at the surface, there is a sharp cutoff between the
adenomatous cells filling the crypt and showing nuclear β-catenin and
surface cells that do not (Fig. 1, K and L). In some areas, however,
the margins were not discrete, and cells with nuclei containing β-catenin
were seen on the surface in continuity with similar cells emerging
from an adjacent crypt (Fig. 1J); however, it is noteworthy that these
adjacent crypts are filled with dysplastic cells containing nuclear
β-catenin, and not merely confined to the upper portions of the crypts.

On the other hand, in larger adenomas, there is unequivocal evidence
of surface cells growing down and replacing the epithelium of normal-
looking crypts (Fig. 1H).

These changes are frequently associated with increased cell prolif-
eration (Fig. 1I), but the proliferative activity, as evidenced by MIB-1
nuclear staining, is distributed throughout the adenomatous crypts and
not confined to the upper parts.

Early Sporadic Adenomas: Microdissection Studies. Crypt fission
was rare in normal and noninvolved mucosa and began with basal
bifurcation at the base of the gland (Fig. 2A). In adenomas, fission was
mostly asymmetrical (Fig. 2B). Budding from the superficial and
mid-crypt was commonly seen (Fig. 2B), in contrast with normal
controls, in which all budding was basal, multiple fission events were
commonly observed in adenomas (Fig. 2C). The crypt fission index
(proportion of crypts in fission) for adenomas at 48% was signifi-
cantly greater than that for noninvolved mucosa (3%). This difference
was significant (P < 0.001).

FAP Adenomas: Histological Studies. The earliest lesion identi-
fied in FAP is the monocryptal adenoma, a crypt that is completely filled
with adenomatous epithelium (Fig. 2D). In the material examined from
the three FAP patients, more than 100 adenomas (both monocryptal and
established) were observed. In some instances, it can be seen that where
the adenomatous crypt is relatively straight and sections are well orien-
tated, the adenomatous epithelium extends to the crypt base, in accord
with our previous three-dimensional observations (13). Moreover, in
these monocryptal lesions, the junction between the adenomatous cells
and the surface mucosa is sharp (Fig. 2, F and G). More developed
adenomas showed evidence of crypt fission and budding (Fig. 2E).

FAP Adenomas: In Situ Hybridization Studies. In the XO/XY
individual, more than 12,000 crypts were examined, and all were
either Y chromosome positive (XY crypts) or Y chromosome negative
(XO crypts). No mixed crypts were found at patch borders in over
1,000 crypts counted, and hence the colonic crypts are clonal (9).
Individual adenomas were small, not exceeding 2.5 mm in diameter.

Fig. 3A shows 1 of the 93 monocryptal adenomas we examined in
this patient, and Fig. 3B shows such a monocryptal adenoma stained
for the Y chromosome: all nuclei are positive. These monocryptal
adenomas all showed either the XO or XY genotype, with no mixed
monocryptal adenomas being seen. Thus all such lesions are clonal
proliferations, as would be expected from the observation that all
crypts are clonal (9). Fig. 3C shows a low-power photograph of a
microadenoma from this patient, with several adenomatous crypts
filled with atypical cells (Fig. 3D). Fig. 3E, of a serial section, shows
that the nuclei of some adenomatous crypts stain for the Y chromo-
some (Fig. 3G), whereas others are negative, except for an occasional
infiltrating lymphocyte (Fig. 3H). Thus this microadenoma is poly-
clonal, composed of adenomatous crypts of both XO and XY pheno-
type; in fact, 76% of adenomas in this patient were polyclonal (9).

Importantly, Fig. 3F, an enlarged field from Fig. 3E, shows an XY
adenomatous crypt approaching the mucosal surface, in immediate
juxtaposition to the point at which an XO adenomatous crypt also
emerges at the surface. Note that the Y chromosome-positive nuclei
stop sharply at the surface and do not encroach into the XO adenom-
atosus crypt territory, indicating that there is no evidence that adenom-
atosus cells migrate superficially over the surface to occupy other
crypts. Furthermore, in none of the 260 more established adenomas
were we able to demonstrate a mixture of XY and XO cells occupying
the same crypt.

Because parts of the Y chromosome in this individual are dupli-
cated, note that the paint shows 2 dots/nucleus if the section is
appropriately orientated.

3821
DISCUSSION

There are several conclusions made possible by these studies. 
(a) In early sporadic adenomas, adenomatous crypts appear as discrete entities. Adenomatous cells fill the entire crypt and appear to stop sharply at the junction with the surface epithelium. Spread out onto the surface into surrounding nonadenomatous crypts is not seen. Adjacent adenomatous crypts, however, are sometimes joined at the surface by adenomatous cells. This supports expansion by crypt fission, and not top-down spread (see Fig. 1).

(b) Crypts in early adenomas display a proliferative architecture, where proliferating cells are seen throughout the crypt, with a reduction in MIB-1 labeling as the surface approaches (Fig. 1F). This in accord with our observations on the distribution of mitoses in micro-dissected adenomatous crypts (21). This again suggests that crypts in early adenomas are discrete entities and replicate the stem cell/transit amplifying/differentiating cell organization seen in normal crypts and supported by studies on the methylation history of adenomas (24). Such an organization does not support the concept that cells grow downwards from the surface to occupy new, uninvolved crypts.

(c) Adenomatous crypts in such early lesions are the site of remarkable fission and budding activity (Fig. 1, F and G; Fig. 2, B and C), suggesting that the main mode of expansion is by fission of preexisting adenomatous crypts.

(d) The earliest lesion detectable in FAP mucosa is the monocryptal adenoma. Again, there is a sharp delineation at the surface between adenomatous and surface cells (Fig. 2F). Monocryptal adenomas expand by fission (Fig. 2E), and lesions larger than one crypt in size show multiple fission events. The monocryptal adenoma appears to be
a clonal proliferation, consistent with origin in a single clonal crypt (Fig. 3, A and B). Observations on the margins of small FAP adenomas show no evidence of adenomatous cells of one genotype invading crypt territories of another genotype (Fig. 3, E and F).

The overall conclusion from these studies is that the major mode of clonal expansion in early adenomas is by crypt fission or budding. If we regard FAP as a model for sporadic colorectal cancer, then the evolution of the monocryptal adenoma to an early microadenoma by crypt fission would also apply. Whereas monocryptal adenomas in non-FAP patients are of course rare, they have been described (Ref. 8; Fig. 2H), and there is certainly no reason not to propose that the early growth of sporadic adenomas is similar, as Fig. 1, F and G, would suggest. But does top-down spread occur in adenomas? Of course it does: Shih et al. (3) demonstrate this very convincingly, and such appearances are indeed common at the margins of larger sporadic adenomas. In fact, there is evidence that can be interpreted in favor of this mode of expansion in FAP as well. Bjerknes et al. (25) showed that adenomatous crypts in FAP contained two stem cell lines, $APC^{−/−} \text{ and } APC^{−/+}$, although they interpreted this to mean that the $APC^{−/−}$ cells were expanding stochastically within the same crypt, rather than growing in from outside. There is good evidence that such monoclonal conversion occurs as a stochastic process (10).

However, Wasan et al. (13), studying larger FAP adenomas by three-dimensional reconstruction, were able to demonstrate adenomatous crypts in direct continuity with nonadenomatous crypts, which of course would be consistent with top-down expansion.

In addition to the sharp junctions at the top of adenomatous crypts, there are instances where cells with $β$-catenin-containing nuclei are in continuity on the surface (Fig. 1J). However, the contributing crypts are wholly occupied by similar cells, and these are not confined to the superficial aspects of the crypts. This would be consistent with crypts dividing but not totally separating, as suggested by previous three-dimensional studies (see Fig. 5C in Ref. 13).

It is worthwhile asking the question—what are the implications of the top-down and bottom-up proposals for studies of clonality in colorectal adenomas? Herein lies a conundrum. In the top-down
scenario, adenomatous cells, presumably of clonal origin, spread from the putative stem cell niche on the surface of the mucosa laterally and downwards to involve multiple adjacent crypts. Fig. 1C in Shih et al. (3) and Fig. 1H in this article show that many crypts are partially involved by the adenomatous epithelium. Because crypts are clonal units, this would suggest that these lesions would be polyclonal, because of the mixture of clonal crypts and clonal adenoma, although of different clonal derivation. In fact studies from the same laboratory of 50 sporadic and FAP adenomas, using X-linked RFLPs, showed these lesions were monoclonal in origin (26). How can this be?

Recent studies by Novelli et al. (27) have shown that the X-linked patch size in the colon is large and can be in excess of 450 crypts in diameter. So, unless an adenoma grows out over a patch boundary and involves crypts on either side of that boundary, X-inactivation analysis will always show that such lesions are monoclonal.

What about the clonal implications of the bottom-up proposals? Expansion of a clonal monocryptal adenoma (Fig. 2E) by crypt fission would inevitably lead to a monoclonal microadenoma and adenoma. However, studies on the X/OXY individual, who has a mean patch size of 1.48 crypts, further studied here, indicated that some 76% of adenomas were polyclonal (9), a result supported by observations in Rosa26/Min chimeric mice (28). So, how can this be explained? Possible mechanisms include the adenomatous transformation of noninvolved crypts under the influence of transformed crypts (28); in fact, this was the explanation chosen for the observation that normal crypts were apparently seen in direct continuity with transformed crypts (see Ref. 13, and see above).

Adenomatous crypts in sporadic adenomas show superficial similarities to normal crypts in the distribution of their proliferative activity. Observations on other, possibly older adenomas have indicated that maximum proliferative activity is found toward the top of the crypts, with the implication that migration kinetics are reversed, with the flux directed toward the bottom of the crypt (29, 30). These observations are backed up by the finding of increased apoptosis at the bottom of adenomatous crypts (29, 31). Such a distribution could support a top-down scenario. In this respect, perhaps counterintuitively, there is evidence from examining the methylation histories of cells in adenomas for a discrete stem cell architecture (24). We have reported previously that crypt mitotic scores were significantly greater in adenomas compared with noninvolved mucosae and normal controls (21). The crypt fission index (proportion of crypts in fission) for adenomas was significantly greater than that of noninvolved mucosae and normal controls. Moreover, studying the zonal distribution of mitoses in adenomas, noninvolved mucosae, and normal controls, we have shown that in normal colonic mucosae, the mitotic figures were located mainly in zones 1 and 2, that is, the base of the crypts (21). In adenomas, mitoses were almost evenly distributed between the five zones. This does not suggest a concentration of dysplastic cells in the tops of adenomatous crypts, as suggested by Fig. 1C of Shih et al. (3), nor is it consistent with the positional observations of Moss et al. (29), using proliferation markers in histological sections. This difference in our results may reflect the difference in techniques because we observed whole crypts after microdissection, which avoids potential geometric artifact imposed by observing sectioned crypts (32). Our previous work on the distribution of mitoses in the flat mucose of FAP patients failed to demonstrate the upward expansion of the proliferative zone reported previously in histological sections using tritiated thymidine by Lipkin et al. (33).

Where adenomas arise, in respect to the crypt and its component cell populations, is important from the viewpoint of the location of the stem cell and the potential target cells for carcinogens in the colon. In the Shih et al. (3) concept, either the stem cell is located on the surface of the colon or the stem cell is located in the classic site, in the crypt base, but adenomatous transformation is not manifest until the transformed stem cell reaches the surface and clonally expands. Whereas this is not the place to review the evidence, there is very strong support for a stem cell niche in the crypt base (34). On the bottom-up proposal, transformation takes place among the stem cell population in the crypt base, the transformed stem cell expands stochastically, and monoclonal conversion gives the monocryptal adenoma, which itself expands early by crypt fission and later by overgrowing adjacent crypts as well.

Others have also concluded that migrating crypt epithelial cells in the upper part of the crypt are the primary targets for transformation by APC mutation (35), and this has received some experimental support (36). In this latter publication, van der Wetering et al. (36) suggest that at the bottom of the crypt, progenitor cells accumulate nuclear β-catenin and express Tcf target genes as a result of Wnt stimulation from surrounding basal pericyrtal myofibroblasts. In normal crypts, cells that reach the mid-crypt region down-regulate β-catenin/Tcf, resulting in cell cycle arrest and differentiation. Cells that carry a β-catenin or APC mutation do not respond to signals controlling β-catenin/Tcf activity, and these cells continue to behave as crypt progenitor cells in the surface epithelium, giving rise to microadenomas. However, two phenomena that are not addressed by the Shih et al. (3) concept are (a) the monocryptal adenoma in FAP and sporadic cases and (b) where the ramifying and budding tubules seen so commonly in adenomas of all varieties come from. As far as the monocryptal adenoma is concerned, there is no apparent explanation that can be proffered within the Shih et al. (3) proposal: an origin from the surface and migration solely into one crypt do not seem feasible. However, with regard to the second problem, it is possible that after the normal crypt territories are completely filled with adenomatous cells, then budding and fission could be a secondary phenomenon. However, our findings of extensive crypt fission in early adenomas would appear to exclude this possibility.

We conclude that crypt fission is an essential event in the clonal expansion of mutated clones in adenomas. Whereas the morphology of this process is quite distinct, the molecular mechanisms that govern it are far from clear. We further conclude that (a) the initial event in the genesis of colorectal adenomas of both sporadic and FAP adenomas is the monocryptal adenoma, (b) initial growth occurs via crypt fission, and (c) spread into adjacent crypt territories is a later, secondary event.

REFERENCES
Bottom-up Histogenesis of Colorectal Adenomas: Origin in the Monocryptal Adenoma and Initial Expansion by Crypt Fission


Updated version
Access the most recent version of this article at:
http://cancerres.aacrjournals.org/content/63/13/3819

Cited articles
This article cites 35 articles, 14 of which you can access for free at:
http://cancerres.aacrjournals.org/content/63/13/3819.full#ref-list-1

Citing articles
This article has been cited by 28 HighWire-hosted articles. Access the articles at:
http://cancerres.aacrjournals.org/content/63/13/3819.full#related-urls

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, use this link http://cancerres.aacrjournals.org/content/63/13/3819.
Click on "Request Permissions" which will take you to the Copyright Clearance Center's (CCC) Rightslink site.