Smoking Out Reproductive Hormone Actions in Lung Cancer

Jill M. Siegfried

Abstract

Experimental and population-based evidence has been steadily accumulating that steroid hormones are fundamentally involved in the biology of the lung. Both estrogen and progesterone receptors are present in normal and malignant lung tissue, and the reproductive hormones that bind these receptors have a role in lung development, lung inflammation, and lung cancer. The estrogen receptor-β (ER-β) was discovered in the 1990s as a novel form of ER that is transcribed from a gene distinct from ER-α, the receptor previously isolated from breast tissue. Interestingly, ER-β is the predominate ER expressed in normal and malignant lung tissue, whereas inflammatory cells that infiltrate the lung are known to express both ER-α and ER-β. Although there is evidence from animal models for the preferential effects of ER-β in the lungs of females, human lung tumors from males often contain comparable numbers of ER-β–positive cells and male-derived lung cancer cell lines respond to estrogens. Lung tumors from both males and females also express CYP19 (aromatase), the rate-limiting enzyme in estrogen synthesis that converts testosterone to estrone and β-estradiol. Thus, testosterone acts as a precursor for local estrogen production within lung tumors, independent of reproductive organs. This review discusses the recent findings about the biology of the ERs, aromatase, and the progesterone receptor in lung cancer and highlights the ongoing clinical trials and future therapeutic implications of these findings. Mol Cancer Res; 12(1); 1–8. ©2014 AACR.

ER Expression in Lung Cancer

Two different genes encode the estrogen receptor (ER) proteins ER-α and ER-β, and these ER proteins are expressed with different tissue distributions (1). Both ER subtypes bind β-estradiol, the most active form of estrogen, with high affinity. Multiple isoforms of ER-α and ER-β have been reported, including three ER-α isoforms (2) and five ER-β isoforms (3, 4). Two lines of evidence suggest that ER-β is the major functional ER in the lung. First, a large difference in expression of ER-β mRNA and ER-α mRNA was observed in human lung tissue during fetal development (5) and in the adult mouse lung (6), with ER-β being the predominant form. Second, a prominent phenotype of the female ER-β knockout (−/−) mouse is a lung abnormality: at 3 months of age, the lungs of these mice contain a decreased number of alveoli and a reduction in expression of key regulators of surfactant homeostasis (6). By the age of 5 months, both female and male mice show alveolar collapse and alterations in extracellular matrix (7), suggesting that estrogen does have a role in lung homeostasis in males as well as females. Because ER-α knockout mice do not show these changes, they are thought to be due to lack of ER-β protein. A suggestion that ER-β is important in lung tumor formation comes from carcinogenesis experiments using the ER-β−/− mouse. In this model, female but not male offspring were protected against development of lung tumors after in utero exposure to the polycyclic hydrocarbon dibenzochrysene (8). Whether this effect is restricted to protection during development or is a general protective effect of ER-β loss on lung cancer formation is unknown. Estrogen was also shown to induce heightened lung tumor formation in female mice exposed to the tobacco carcinogen benzo[a]pyrene. A 50% increase in tumor multiplicity in the lungs and a 60% increase in lung tumor incidence were induced by β-estradiol (9).

ER-α and ER-β proteins can be distinguished by selective antibodies, and there is general agreement from a series of published studies that the full-length 59 kDa ER-β protein (the ER-β1 isoform) is expressed in most human non–small cell lung cancer (NSCLC) cell lines, and is frequently detected in tissue specimens of human NSCLCs from men as well as women (10–14). ER-β protein is found at higher levels in lung tumors compared with matching normal lung tissue from the same patient (14), suggesting upregulation in cancer. ER-β protein in lung tumors is detected in both nuclear and cytoplasmic compartments, and smaller variants of 53 to 56 kDa are often coexpressed (10). These smaller variants are the ER-β isoforms 2 to 5 (15), which do not have functional activity for classical activation of estrogen response elements (ERE), although they can heterodimerize with ER-β1 and enhance its transcriptional activity (3). It also seems that full-length ER-β1 protein prefers to dimerize with the other lower molecular weight isoforms, so their presence could be a strong modulator of ER-β action (3).
The frequency of expression and function of the different ER-β isoforms in lung cancer is not well understood, because a comprehensive comparison of the five known ER-β isoforms has not been undertaken. Although ER-β isoforms 2 to 5 lack the helix structures that allow for agonist conformation in ER dimers that are required for transcriptional activation, it is unknown whether these isoforms can function in nongenomic signaling pathways (described below) to activate kinases or interact with Akt or other signaling molecules, or if they can modulate nongenomic signaling as heterodimers with ER-β1. The properties of the ER-β isoforms are shown in Table 1. ER-β3 is found only in testis tissues, whereas the other isoforms have been found in breast, prostate, and lung tissues, among others.

Whether there is lung tumor expression of full-length ER-α is controversial. ER-α staining of lung tumor tissues and cell lines was found primarily in the cytoplasm and on the cell membrane, with rare expression in the nucleus (10–14), and both mRNA and protein analysis showed ER-α messages to be comprised of alternatively spliced variants (10). These variant isoforms lack the amino-terminus because the proteins are differentially detected by antibodies that recognize the ER-α amino- and carboxy-terminal (10). Immunoblotting of cell lines failed to detect the expected 66 kD ER-α protein, whereas smaller variants of 42 and 54 kD were found (16, 17). Protumorigenic effects such as estrogen-mediated RNA transcription of proliferation genes, nongenomic signaling that activates tyrosine kinases, and cell division in lung tumor cell lines can all be blocked by the ER inhibitor fulvestrant, demonstrating that ERs found in lung cancer are functional (10, 16, 17). A study of ER-α and ER-β selective agonists shows that biological effects are predominantly mediated by ER-β (17). Although ER-α protein may be detected in a subset of lung tumors in patients, such as those with epidermal growth factor receptor (EGFR) mutation (18), ER-β seems to be the major ER expressed in lung cancer and responsible for mediating protumor effects of estrogen. This is in contrast to breast and colon tissues, in which ER-β is thought to oppose proliferative effects of ER-α. This may be due to differential recruitment of coactivators to ER-β in the lung (16, 17) compared with other tissues, to the absence of full-length functional ER-α protein in most lung tumors, or to the predominance of nongenomic signaling to cytoplasmic growth factor pathways in lung cancer through ER-β.

There are now many published reports examining ER status in relation to NSCLC survival. Nuclear ER-β staining was scored in the majority of lung cancer cases (11–14, 19) and found to be associated with favorable prognosis in some studies. The prognostic significance was often limited to a subset of patients with a particular mutation, or only observed in male patients (12–14). However, most studies utilized antibodies to total ER-β that could not distinguish different ER-β isoforms. Recently, high cytoplasmic ER-β1 staining was identified as an independent negative prognostic factor for lung cancer (19). The negative effect on survival of ER-β1 was observed in male and female patients and showed no interaction with sex. Prognostic significance of cytoplasmic ER protein may be related to the importance of nongenomic signaling for ER action in the lung. Isoform specificity was also reported in a study demonstrating that ER-β1, but not ER-β2, was related to worse prognosis in female patients with stage I lung cancer (20). Nuclear ER-β1 also correlated with poor survival in metastatic lung cancer, but not patients with early-stage lung cancer (21). In contrast, the ER-β2 and -β5 isoforms have been linked to better lung cancer outcome (15), although the mechanistic basis for this effect is unknown.

There is no consensus on survival effects of differences in expression of ER-α protein in lung tumors, which as noted above is predominantly found as smaller variant proteins. It is variously reported that ER-α has no effect on survival, or to correlate with poor prognosis (13, 14, 19). Nuclear and cytoplasmic ERs may have distinct functions and each component should be assessed both separately and together in tissue specimens of patients with lung cancer. Additionally, the isoform type may be critical to interpreting survival data. A growing literature also shows that ERs localize to mitochondria and that estrogen can induce expression of the mitochondrial genome as well as increase vulnerability to oxidative stressors such as hydrogen peroxide. Recently, reports of mitochondrial action of ER-β in lung cancer cells have suggested it seems to protect against apoptosis (22) and to show reduced activity during allergic airway inflammation in a mouse model of asthma (23). Analysis of the different ER-β isoforms as well as their cellular localization will be necessary to completely understand the role of ER-β in lung cancer. If standardized approaches can be developed, these hormone receptor markers may become useful biomarkers, potentially able to predict the aggressiveness of lung cancers and to identify patients who might be candidates for hormonal therapy.

**ER Signaling in Lung Cancer**

ERs belong to the nuclear steroid receptor superfamily, and signal to produce cellular responses following binding of estrogenic hormones (24). ERs function through several different mechanisms. As transcription factors, ERs bind EREs and recruit coactivators that facilitate gene transcription. The ability of ERs to bind EREs is dependent upon ER activation, which occurs following ligand binding. Evidence that the lung is an estrogen-responsive tissue was seen in the transgenic ERE-luciferase reporter mouse, which used
luciferase expression as an indicator of ERE activation following estrogen treatment. A 15-fold induction of reporter gene expression following treatment with estrogen was observed in the lungs of both male (25) and female (26) mice. ERs can also be activated through kinase phosphorylation, a process that does not require estrogen binding, and the phosphorylated ER functions as an estrogen-independent transcription factor (1). Growth factors that activate tyrosine kinase receptors such as EGFR or insulin-like growth factor I receptor (IGF-1R) can initiate signaling to downstream kinase pathways, which subsequently phosphorylate the ER and allow it to signal independently of estrogen. For example, EGFR can directly phosphorylate ER at specific serine residues (27). These residues were found to be phosphorylated in 87.5% of ER-positive lung tumors examined, suggesting that EGFR activation of ER is common in lung cancer (28).

The nuclear actions of ERs involve changes in gene transcription that take place over several hours or longer through direct binding of ERs to promoter elements of estrogen response genes. However, estrogen can also rapidly activate cytoplasmic kinase signaling in seconds to minutes. This rapid signaling, termed nongenomic, occurs via non-nuclear ERs located in the membrane or the cytoplasm. In breast cancer cells, an additional membrane ER was identified as a G protein–coupled receptor called GPR30 (29). Expression of GPR30 has recently been demonstrated in lung cancer cells but the function and regulation of GPR30 in the lung is still unknown (30), and some data suggest that GPR30 exists in a complex with an ER and is not an independent receptor (31). In NSCLC cells, extranuclear ERs have been identified in plasma membrane fractions and cytoplasmic fractions. Treatment with estrogen or ER-β–specific ligands has been shown to promote stimulation of tyrosine kinase signaling pathways within 5 to 10 minutes (17, 32). These effects can be inhibited by the addition of fulvestrant, an ER antagonist.

Nongenomic ER signaling occurs via activation of tyrosine kinase growth factor pathways, such as the EGFR/human EGF receptor (HER)-1 and the IGF-1R. EGFR is a member of the tyrosine kinase receptor family that also includes HER-2, HER-3, and HER-4 (33), and many lung tumors are highly dependent on these pathways for growth and survival (34). In lung cancer cells (28, 32, 35), estrogen can rapidly activate the EGFR through release of EGFR ligands, and the combination of the antiestrogen fulvestrant and an EGFR tyrosine kinase inhibitor (TKI) such as gefitinib or erlotinib can maximally inhibit cell proliferation, induce apoptosis, and reduce downstream signaling pathways both in vitro and in vivo (35, 36). Erlotinib, the EGFR inhibitor that is approved by the U.S. Food and Drug Administration for NSCLC, gave the best antitumor effects in NSCLC tumor xenografts when combined with fulvestrant (35, 36). The multitargeted TKI, vandetanib, which targets EGFR and VEGFR, also showed additive effects when combined with fulvestrant (37). A synergistic effect of gefitinib combined with the reversible nonsteroidal aromatase inhibitor, anastrozole, was also observed in lung cancer cell lines, further suggesting a functional interaction between EGFR and ER pathways (38). Additionally, membrane ERs were colocalized with EGFR in lung tumors (32).

Aromatase in Lung Cancer

CYP19 (aromatase), a member of the cytochrome P450 family, catalyzes the conversion of androstenedione and testosterone to estrone and β-estradiol, respectively. Both CYP19 mRNA and protein have been detected in the lung (39, 40). High β-estradiol levels were detected by mass spectroscopy in intratumoral extracts of primary NSCLC (41), and β-estradiol and its metabolites have also been detected in cell lines derived from lung tumors (42). Aromatase protein was detected in both NSCLC cell lines and tumor tissues, and the aromatase-positive NSCLC cell lines were shown to secrete β-estradiol (43). The aromatase inhibitor anastrozole caused a substantial inhibition in growth of aromatase-positive NSCLC tumor xenografts (43). Anastrozole was also demonstrated to prevent lung carcinogenesis in female mice exposed to a tobacco carcinogen; this effect was further increased by the addition of the ER antagonist fulvestrant (44). Interestingly, in this animal model of lung cancer prevention, aromatase expression was localized almost exclusively to macrophages and plasma cells that infiltrated preneoplastic and neoplastic areas of the lungs. Some aromatase protein localized to the abnormal epithelium, but at reduced intensity compared with the inflammatory cells (44). An important source of estrogen synthesis may therefore be pulmonary inflammatory cells that infiltrate the lungs in response to carcinogens. This local production of estrogen may be part of the chronic inflammatory reaction occurring throughout the process of lung cancer development. Coombes and colleagues reported a lower incidence of primary lung cancer in patients with breast cancer who were treated with the aromatase inhibitor exemestane following several years of tamoxifen therapy compared with those women who continued tamoxifen treatment (45). This suggests that aromatase inhibitors could suppress the development of lung cancer in postmenopausal women.

Mah and colleagues (46) found aromatase to be a predictive biomarker of lung cancer survival in older women with early-stage lung cancer. Women over age 65 with lower levels of aromatase in tumor tissue had a greater chance of survival compared with those with higher aromatase expression. The prognostic value of aromatase expression was greatest in patients with stage I and II lung cancer. In this postmenopausal population of patients whose circulating estrogen levels are low due to decreased production by the ovaries, local estrogen production through tumor expression of aromatase could be an important determinant of estrogen levels. In a separate study that included all stages of lung cancer, no general association between aromatase and lung cancer survival was observed unless combined with other markers such as ER-β, EGFR, and progesterone receptor (PR) expression (19). No effect of sex or menopausal status was found for aromatase in this study. However, the study did not focus on older postmenopausal women. On the
PR in Lung Cancer

The two major isoforms of PR, PR-A and PR-B, play different roles in modulating cellular responses to progesterone. PR is an estrogen response gene, and PR-positive breast cancers are usually more differentiated tumors that respond to antiestrogen therapy. The ratio of PR-A:PR-B is known to signal through ligand-inhibit migration and invasion of lung cancer cell lines (54). One mechanism for low tumor PR expression in breast cancers is through increased growth factor signaling, which leads to a more aggressive tumor biology with faster progression (56). Whether or not this same mechanism occurs in lung tumors is unknown and is currently being investigated.

Progesterone derivatives have been useful in the treatment of both endometrial cancer and breast cancer (57, 58). Agents such as medroxyprogesterone acetate, which can be given orally, have potential for treatment of lung cancer, perhaps in combination with agents that suppress either the ER pathway or act on growth factor pathways such as EGFR, c-Met, or other TKIs. Whether progesterone could be used for prevention is open to debate because it can also have angiogenic properties.

Reproductive Hormones and Lung Cancer Survival

It is well established that women with advanced NSCLC live longer than men (59), although this observation is not specific to lung cancer, but is found in many tumor types. How much of this survival difference might be attributed to hormonal differences is not clear, and whether this survival difference is caused by differences between men and women in how cancer progresses, or to processes that generally lead to longer lifetimes for women compared with men is also unknown. A study seeking to address hormonal aspects of survival examined lung cancer presentation in pre- versus postmenopausal women. The results showed more advanced disease including poorly differentiated tumors with less favorable histologies occurred more often in premenopausal than postmenopausal women (60). Despite this, a significant survival difference between pre- and postmenopausal women was not seen. In a related study, women over the age of 60 had a significant survival advantage over both men and younger women, a difference potentially attributable to hormonal status because men did not show survival differences by age (61).

Several studies have now reported that exposure to hormone replacement therapy (HRT) was associated with negative effects on lung cancer survival. Ganti and colleagues (62) reported a significant association between both a lower age at lung cancer diagnosis and a shorter survival time in women who used HRT around the time of diagnosis versus those who did not. This effect was more apparent in women who smoked, suggesting an interaction between estrogens and tobacco carcinogens. In a randomized, placebo-controlled trial in which more than 16,000 postmenopausal women received placebo or daily HRT for 5 years (the Women’s Health Initiative), a strong negative effect on survival after a lung cancer diagnosis was observed in women on the HRT arm (63). The HRT group had a significantly greater likelihood of dying from lung cancer compared with placebo, group with a trend toward more lung cancer diagnoses in the HRT arm. A role for estrogen in lung cancer presentation is supported by several retrospective population studies demonstrating that antiestrogen use improves survival of female patients with lung cancer. An observational study, which included more than 6,500 breast cancer survivors, found that women who received any antiestrogen treatment had significantly lower subsequent lung cancer mortality (64). The Manitoba Cancer Registry also evaluated 2,320 women with or without exposure to antiestrogens (65). Antiestrogen use both before and after lung cancer diagnosis was significantly associated with decreased lung cancer mortality. Published studies on both
HRT and antiestrogen use support the idea of estrogen acting as a promoter of lung cancer aggressiveness that may play a key role not only in the biology but also the outcome of lung cancer.

Effects of hormone replacement that promote poor outcomes in lung cancer could be due to both direct effects on tumor proliferation and indirect effects caused by response of the microenvironment to estrogen. For example, endothelial cells show greater survival in the presence of β-estradiol (66), an effect that could increase angiogenesis. ER-β was critical for progression of lung cancer metastasis in the brain, due in large part to a contribution from astrocytes that express ER-β (67). Estrogen and progesterone mediated resistance of lung cancer cells to apoptosis induced by cisplatin, a major chemotherapy drug used to treat lung cancer (68).

Reproductive Hormones and Lung Cancer Risk

Although many studies suggest that exposure to exogenous hormones is detrimental to survival of patients with lung cancer, whether exogenous hormone use before diagnosis affects the risk of developing lung cancer is controversial. In the Women’s Health Initiative study described earlier, the HRT arm had an increase in incidence of lung cancer compared with placebo, but it was not statistically significant (63). Two other large cohorts with HRT exposure data have also been examined for relationship to risk of a later lung cancer. The Vitamins and Lifestyle Study followed 36,000 postmenopausal women who filled out baseline questionnaires about hormone use, and were assessed for lung cancers diagnosed up to 7 years after baseline data were collected. The California Teachers Study consisted of over 60,000 postmenopausal women who supplied baseline data about hormone use and were assessed for lung cancer diagnoses up to 11 years after baseline. In contrast to the Women’s Health Initiative, a significant increase in lung cancer incidence associated with HRT was observed in the Vitamins and Lifestyle Study, and this effect on lung cancer risk was duration dependent (69), whereas in the California teachers cohort, no effect of HRT on lung cancer risk was observed (70). Two other case–control reports suggest that HRT use before diagnosis could protect women from developing lung cancer (71, 72). In one study, a protective effect was especially found in women who smoked (71), and an inverse relationship was also observed between HRT use and NSCLC risk in postmenopausal women with ER-positive, but not ER-negative lung tumors (72). A recent pooled analysis of six case–control studies (1,961 cases and 2,609 controls) from the International Lung Cancer Consortium found a strongly reduced lung cancer risk associated with both oral contraceptive use and HRT use, independent of smoking status or body mass index (73).

It is interesting that case–control studies show protective effects of exogenous hormone use, whereas cohort studies do not, or show detrimental effects. In the pooled case–control analysis (73), neither duration of use nor dose were factors in the degree of protection, suggesting short exposures were equally protective, whereas in cohort studies a longer duration was more detrimental for either lung cancer incidence or survival. There could be differences in the balance between procarcer and anticancer effects of estrogen in normal lung epithelium compared with malignant epithelium that could change over time. ER-β is overexpressed in lung tumors compared with matched normal lung tissues (19), which could lead to abnormal responses to estrogen in malignant cells that promote lung cancer. ER-β expression is induced by oxidative stress and inflammation in both endothelial cells and macrophages, which could also lead to procarcer responses to estrogens in areas of the lung that are at risk for lung cancer development. However, the immune system is also regulated by estrogen, and the ability of the immune system to reject malignant lung tissues early in the cancer process could be enhanced by hormone use. Depending upon context, estrogen can either suppress or enhance inflammation, and estrogen can also down-modulate the release of other growth factors that contribute to cancer development. Because lung tumors are known to produce aromatase, it is possible that exogenous hormone use reduces estrogen production in the airways by inhibiting pulmonary aromatase expression. This could also repress production of hydroxyl forms of estrogen, which are only produced in the periphery, and which also have pro- and anticancer effects (74). The balance of these processes may contribute to whether hormone use results in protection from or promotion of lung cancer. Because it is now recommended that HRT use be of limited duration in postmenopausal women, due to general health hazards of long-term use, HRT effects on lung cancer risk or outcome may be less pronounced in the future.

Clinical Trials Using the ER Antagonist Fulvestrant in Lung Cancer

The body of evidence showing proliferative effects of estrogen on lung tumors and an interaction between ER and EGFR signaling in lung cancer suggests that targeting both pathways could be beneficial for therapy. Targeting the EGFR using TKIs as a single therapy is of limited use in the absence of an EGFR mutation, which occurs in about 20% of patients with adenocarcinoma. The patients who respond well to EGFR TKIs are mainly females and never smokers (75), which may relate to cross-talk in signaling between the EGFR and ER in lung cancer (76). As noted above, some studies have reported a correlation between EGFR mutation and ER expression (18, 76). Knowledge of ER-EGFR cross-talk was translated in a phase I clinical trial using drugs that target EGFR and ER, that assess the toxicity of combined treatment of gefitinib with fulvestrant (77). Targeting both pathways was found to be safe and to have antitumor activity in female patients with advanced, pretreated NSCLC. Additionally, high ER-β expression was correlated with better patient survival in this cohort of fulvestrant-treated patients. A phase II trial examining the combination of erlotinib (the now-preferred EGFR TKI) with fulvestrant compared with erlotinib alone has recently been completed, in which 100 patients were treated (78). Combination treatment was well tolerated. Progression-free survival and response rate were

[www.aacrjournals.org](www.aacrjournals.org) Mol Cancer Res; 12(1) January 2014 OF5
similar between the two treatment arms in unselected patients. However, among patients with EGFR wild-type tumors, the clinical benefit rate (which included partial responders and those with stable disease) was significantly higher among patients treated with the combination compared with erlotinib alone, with trends toward improved survival. It is yet to be determined what ER-related biomarkers will be informative in defining the patients most likely to benefit. These clinical trials suggest that targeting the ER pathway in conjunction with the EGFR pathway, or other aberrantly expressed tyrosine kinase receptors, will have beneficial antitumor effects in NSCLC as has been observed in breast cancer cells (79), particularly in patients whose tumors do not contain an EGFR mutation. The combination of antiestrogen therapy or aromatase inhibitors with targeted therapies that block different growth factor receptors also warrants clinical investigation.

Summary

A body of preclinical evidence now demonstrates that estrogen is a driver of lung cancer. Estrogens induce cell proliferation of NSCLC cells in cell culture (10, 32), human tumor xenografts (10), and in animal models of lung cancer (9, 80). Estrogen can also modulate expression of genes in NSCLC cell lines that are important for inducing cell proliferation such as c-myc and cyclin D1 (16). Estrogen signaling through ERE and AP-1 promoter elements was shown to occur primarily through ER-β in NSCLC cells, as was enhanced lung tumor xenograft growth (17, 35). In addition, fulvestrant, a pure ER antagonist, inhibits proliferation in NSCLC cell lines and in lung tumor xenograft models in immunocompromised mice (10). This preclinical evidence combined with population data showing that HRT use reduces lung cancer survival strongly support targeting the ER pathway therapeutically. Several ongoing trials seek to find biomarkers that will identify the patients with lung cancer most likely to benefit from abrogation of hormone pathways.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Grant Support

This study was partially supported by NCI Grant P50 090440, SPOR in Lung Cancer, and by the V Foundation for Cancer Research.

Received November 4, 2013; revised December 9, 2013; accepted December 11, 2013; published online January 17, 2014.

References

20. Sethi S, Coti M, Lonardo F. Expression of estrogen receptor beta 1 but not estrogen receptor beta 2 or alpha is linked to worse prognosis in stage I adenocarcinoma, in women, in a large epidemiological cohort but not in a smaller, single hospital based series. United States and Canadian Academy of Pathology; 2010. Abstract 1843.
Reproductive Hormone Action in Lung Cancer


Smoking Out Reproductive Hormone Actions in Lung Cancer

Jill M. Siegfried

Mol Cancer Res  Published OnlineFirst January 7, 2014.

Updated version  Access the most recent version of this article at:
doi:10.1158/1541-7786.MCR-13-0580

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.