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Gender, *p53*, and Lung Cancer

By Jennifer E. Tseng, MD, and Li Mao, MD

Lung cancer is the leading cause of cancer death in both men and women, with an estimated incidence of 164,100 new cases of lung cancer in the United States in 2000, and an estimated 156,900 deaths.[1] The rate of rise in the incidence of lung cancer in women has increased rapidly; in the 30-year period ending in 1985, there was a 396% increase in the incidence of lung cancer in women compared to a 161% increase in men.[2] In recent years, lung cancer mortality in men has decreased at the rate of -1.6% per year between 1990 and 1996, while the mortality rate of lung cancer in women has only recently begun to plateau.[1] Previous authors have reported that women with lung cancer were more likely to be diagnosed at an early age than men with lung cancer, more likely to be lifetime non-smokers, and, in patients who have a positive tobacco history, to smoke fewer cigarettes per day than males with lung cancer.[2,3]

Background

Thompson et al, in a recent analysis of 1044 patients with lung cancer, reported that a significantly higher proportion of females with lung cancer had small cell histology compared with men.[4] This gender difference was more striking in younger patients; 34% of females younger than 65 years with lung cancer had small cell lung cancer (SCLC) compared to 18% of men.[4] Furthermore, Ferguson et al found that women with SCLC were significantly younger than men with SCLC at the time of diagnosis, with a mean age of 57.4 years for women compared with 60.2 years for men.[4] Zang et al demonstrated a higher risk of lung cancer in females when controlled for cumulative tobacco exposure.[5] Potential biological mechanisms for this gender difference include differences in nicotine metabolism, gender differences in cytochrome p450 enzymes, and hormonal influences on tumor development.[5,6] Bennett et al recently investigated mechanisms of genetic susceptibility in a population of women who had never smoked and found that women with exposure to environmental smoke who developed lung cancer were more likely to be deficient in glu-

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tathione S-transferase M1 due to a germline polymorphism in the *GSTM1* gene.[7]

Intriguingly, women with SCLC have an improved survival rate compared to men with SCLC in multiple studies.[2,8-10] Similarly, O'Connell et al found that females with non-small cell lung cancer (NSCLC) had a significantly improved survival compared with men, with median survivals of 12.4 months vs. 8.8 months, respectively.[11] The biological reasons for these differences between males and females with lung cancer are unclear. It is possible that gender differences in susceptibility to genetic mutations are at least partially responsible for these biological differences.

Mutation in the *p53* tumor suppressor gene is one of the most frequently found genetic abnormalities in human cancers. The *p53* gene has been found to be mutated in a significantly higher proportion of SCLC patients compared to NSCLC (70% vs 47%, respectively).[12-17] However, this conclusion was mainly based on data derived from cell lines or biased gender populations, and included small sample sizes. Previous authors have reported a trend toward a higher frequency of *p53* mutations in males with NSCLC compared to females.[18] In a meta analysis of 1674 patients with NSCLC for whom gender data was available, *p53* alterations (protein expression and/or mutation) were more frequent in males compared to females (48.3% and 32.1%, respectively).[19] However, previous studies have not detected a significant gender difference in *p53* mutational frequency in SCLC, as few studies have included significant numbers of female patients with SCLC.

Primary SCLC Tumors Analyzed for *p53* Mutation Status

Recently, we reported a study in which primary SCLC tumors from 65 patients (38 males and 27 females) were analyzed for *p53* mutation status in order to reveal a potential gender difference.[20] The mean age of this study population was 58.9 years. There was no significant difference between males and females in this series in mean age, race, or response to chemotherapy. There were non-significant trends toward greater tobacco exposure in males compared to females (72 pack-years vs 59 pack-years, respectively), heavier alcohol consumption in males compared to females, and better performance status at diagnosis in females compared to males. The only clinical variable in this series that was significantly associated with gender was stage of disease, with a higher proportion of females presenting with limited stage disease at the time of diagnosis. There was no significant association between *p53* mutational status and survival, age, race, cumulative tobacco exposure, and response to chemotherapy. There was a non-significant trend toward a higher frequency of *p53* mutations in patients with extensive disease stage, heavy alcohol consumption, and worse performance status. There was no significant association between overall survival and *p53* mutational status. After adjusting for disease stage, there was also no significant association between gender and frequency of *p53* mutation.

Thirty-seven (57%) of the 65 tumors were found to have one or more mutations, with a total of 42 mutations.[20] There was a trend toward a higher frequency of *p53* mutations in tumors from males; among tumors from females, 46% contained *p53* mutations, compared to 65% of 37 tumors from males. Furthermore, tumors from four (17%) of the 24 males had more than one mutation in the *p53* gene, compared to none of the tumors from females. Mutations were demonstrated throughout all exons analyzed; the majority of mutations (81%) were within exons 5 to 7. Thirty-one of 42 mutations (74%) were missense mutations resulting in the substitution of one amino acid for another. The remaining mutation types were as follows: 10% were small deletions or insertions causing frameshifts in coding for the p53 protein, 5% were silent mutations, 5% were located at intron/exon boundaries, and 5% were non-

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sense mutations resulting in the substitution of a termination codon.

Presenting Nucleotide Substitutions

The most frequent type of nucleotide substitution in both males and females was the A:T→G:C transversion, comprising 29% of all tumors, 23% of primary tumors in females, and 35% of tumors in males.[20] The second most frequent nucleotide substitution overall was the G:C→T:A transversion, comprising 17% of all tumors and 21% of tumors in males but only 8% of tumors in females. In females, G:C→A:T transitions and A:T→T:A transversions were both more frequent than G:C→T:A transversions, each comprising 15% of the total. Other types of nucleotide substitutions were present in less than 10% of tumors. These differences in the patterns of nucleotide substitutions in males and females were not statistically significant.

The spectrum of nucleotide substitutions in SCLC primary tumors has been demonstrated to be more diverse than that in NSCLC primary tumors.[16] In our study, A:T→G:C transitions were the most common mutation (29% of all mutations), followed by G:C→T:A transversions (17%) and G:C→A:T transitions (12%). Previous studies have demonstrated an association between patterns of nucleotide substitutions and specific mutagens involved.[21-24] G:C→T:A transversions are associated with benzo[a]pyrene,[21,23,24] while A:T→G:C transitions may be induced by N-ethyl-N-nitrosourea.[22] In a review of *p53* mutations of 92 SCLC cell lines and primary tumors, Greenblatt et al found that G:C→T:A transversions comprised 46% of the mutations while A:T to G:C transitions occurred in only 9%.[16] Intriguingly, a high rate of A:T to G:C transitions in SCLC has previously been demonstrated only in a Japanese population.[12] Our study was the first to demonstrate that A:T to G:C transitions in the *p53* gene occur frequently in an American population with SCLC. It may be that the patients included in our study have been exposed to or are susceptible to carcinogens such as N-ethyl-N-nitrosourea, which are similar to those of Japanese patients included in the study by Takahashi et al.[12]

Summary

To our knowledge, this is the largest study to date analyzing *p53* mutations in SCLC primary tumors which has included a significant number of female patients with SCLC. We have demonstrated a trend toward lower frequency of *p53* mutations in primary SCLC tumors of females compared to males, suggesting that males with SCLC may be more susceptible to certain carcinogens in tobacco smoke that preferentially induce mutations in *p53*. This difference is quite intriguing, as it may partially explain biological differences in tumors of males and females with SCLC. Among patients with limited disease, females have a significantly higher rate of complete response than males.[10] Given the increased risk for the development of SCLC in females, the improved survival of females with SCLC is somewhat surprising. The lower frequency of *p53* mutations in SCLC tumors from females may be one possible mechanism for this improved survival. Although our study did not demonstrate a significant association between *p53* mutation status and survival, this may be due to the small sample size of patients studied. It would be important to evaluate *p53* mutational frequency in larger populations of patients with small cell lung cancer in order to determine whether this trend toward lower frequency of *p53* mutations in females is confirmed in larger series. *(Dr. Tseng is a Fellow, Medical Oncology, and Dr. Mao is an Associate Professor of Medical Oncology at M.D. Anderson Cancer Center, Houston, TX.)* ❖

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Paracrine Regulation of Osteoclast Activity in Metastatic Breast Cancer

By A. Shaw and M. J. Oursler, PhD

Bone is the most common site of breast cancer metastasis. Breast tumors in the bone marrow cavity recruit osteoclasts to degrade bone, forming localized areas where bone is considerably weakened. Metastatic breast cancer-induced bone loss is termed osteolysis. Osteolysis leads to hypercalcemia and bone fractures, resulting in considerable pain for patients with metastatic breast cancer. Elevated bone resorption by osteoclasts is responsible for tumor-induced osteolysis. The mechanism of the increase in osteoclast activity that causes osteolysis is believed to involve soluble growth factors secreted by the tumor. Recently, the importance of these paracrine influences on osteoclast activity have been recognized, and it is the purpose of this review to discuss the results of investigations into the mechanism of tumor-derived growth factor actions on osteoclasts.

Introduction

Paracrine factors are implicated as mediators of bone loss associated with tumor osteolysis in patients with metastatic breast cancer. Osteolytic lesions result from an increase in osteoclast bone resorption activity at sites adjacent to a tumor in the marrow cavity.[1] Bone loss during tumor osteolysis may result from any of the following mechanisms: 1) increased formation of osteoclasts; 2) increased resorption activity by mature osteoclasts; or 3) increased survival of mature osteoclasts.

Osteolytic bone loss may result from increased formation of mature osteoclasts from osteoclast precursors. Mature osteoclasts form by differentiation from hematopoietic stem cells found in the marrow activity. This process requires direct cell-cell contact between osteoclast precursors and marrow stromal cells. Later stages in osteoclast differentiation include fusion of mononuclear osteoclast precursors to form multinucleated cells, and activation of multinucleated osteoclasts to induce adhesion to bone and secretion of bone-degrading enzymes.[2]

Osteolytic bone loss may result from an increase in the resorption activity of each individual osteoclast. Bone resorption activity is related to the ability of an osteoclast to adhere to bone, secrete bone-degrading lysosomal enzymes, and migrate to form more and/or larger resorption

pits on bone slices. A mature osteoclast may be activated by a number of stimuli. Upon activation, an osteoclast will adhere to bone, forming a tight-sealing zone around the periphery of the bone resorption compartment, which is analogous to a secondary lysosome. Bone-degrading proteases are secreted into this compartment by fusion of lysosomal vesicles with the plasma membrane adjacent to the bone surface. Fusion of these vesicles increases the amount of membrane at this interface and this portion of the plasma membrane becomes highly convoluted, forming a structure called the ruffled border of the osteoclast.

The bone resorption compartment becomes acidified by the action of numerous proton pumps on the ruffled border. The combined action of acid and proteases degrades the bone surface enclosed by the sealing zone. An active osteoclast may detach, migrate, and reattach at another site to form multiple resorption pits. The important role that soluble growth factors play in activation of osteoclasts in metastatic breast cancer was first alluded to by a study that examined the conditioned medium of an osteolytic breast cancer cell line, MDA MB 231.[3]

This study revealed that 231 cell conditioned medium was capable of increasing the activity of mature osteoclasts, suggesting that the medium contained soluble factors that could act on osteoclasts. Analysis of the 231 cell conditioned medium revealed that it contained many growth factors that have been implicated as regulators of osteoclast activity. This conditioned medium was fractionated over a sizing column and used to treat osteoclasts. Some fractions stimulated, while some fractions inhibited osteoclast activity. This observation suggests that stimulatory factors overcome the actions of inhibitory factors to increase osteoclast activity. Paracrine stimulation of osteoclast activity may be a primary mechanism by which metastatic breast tumors are able to degrade bone.

Osteolytic bone loss may result from increased survival of mature osteoclasts. It is believed that mature osteoclasts are removed from the bone surface by a signal to undergo apoptosis. Soluble factors secreted by tumors may delay the apoptotic signal, allowing osteoclasts to continue with their bone resorption program for a longer period of time. Below, several paracrine factors that may be important in the development of osteolytic lesions are discussed.

OPG/RANKL/RANK

Osteoprotegerin (OPG) is a recently discovered protein that plays an important role in osteoclast formation. OPG is a soluble factor secreted by bone marrow stromal cells and osteoblasts. OPG functions as an inhibitor of osteoclast formation. OPG interferes with a critical interaction between the osteoclast precursor receptor-activator of NFkB (RANK) and its cognate ligand (RANKL). RANK is present on the plasma membrane of osteoclast precursors and mature osteoclasts, while RANKL is expressed on the plasma membrane of stromal cells. In addition, the interaction between RANKL and RANK gives rise to signaling events that inhibit apoptosis in mature osteoclasts. In this way, OPG reduces overall osteoclast numbers by blocking osteoclast formation and stimulating apoptosis of mature osteoclasts. Conversely, increased levels of RANKL are correlated with increased osteoclast numbers. One might expect that osteolytic tumors would express RANKL or induce RANKL expression. However, we and others have shown that most primary breast cancers, metastatic breast cancers, and breast cancer cell lines do not express RANKL.[4] When breast cancer cells are co-cultured with bone marrow stromal cells, expression of RANKL is induced and OPG is inhibited.[5] These results indicate that another factor produced by breast cancer cells within the bone marrow cavity induces RANKL expression and inhibits OPG secretion by bone marrow stromal cells.

Macrophage-Colony Stimulating Factor

Macrophage-colony stimulating factor (M-CSF) is absolutely required for osteoclast formation. M-CSF is expressed by bone marrow stromal cells and osteoblasts. The essential role of M-CSF is evident in mutant op/op mice that express nonfunctional M-CSF. These mice show a complete lack of mature osteoclasts, a condition that is reversed by infusion of M-CSF. M-CSF stimulates proliferation of early osteoclast precursors and induces migration of actively resorbing osteoclasts. Estrogen deficiency enhances M-CSF production, which results in the overall increase in osteoclast number seen in osteoporosis. M-CSF also promotes the survival of mature osteoclasts by delaying the onset of apoptosis. M-CSF and RANKL have been shown to be essential factors for osteoclast formation in vitro.[6]

Granulocyte-Macrophage Colony Stimulating Factor

Granulocyte-macrophage colony stimulating factor (GM-CSF) has opposing effects on osteoclast formation. Effects of GM-CSF appear to depend on the differentiation state of the target cell. GM-CSF stimulates proliferation of early osteoclast precursors, but potently inhibits late stages of osteoclast differentiation. GM-CSF inhibits formation of osteoclasts from mouse bone marrow, which contains mid-stage osteoclast precursors. In an in vivo nude mouse model of osteolysis, expression of GM-CSF declined as osteolytic lesions appeared.[7] Local repression of GM-CSF to allow osteoclast formation may provide a mechanism by which an osteolytic tumor can mediate bone loss.

Tumor Necrosis Factor Alpha

Tumor necrosis factor alpha (TNF-α) is a soluble protein secreted by many bone marrow cells and is known to play a role in the development of other bone loss pathologies, including periodontitis and orthopedic implant loosening. Antibody blockade of TNF-α results in decreased osteoclast numbers and reduced pit formation activity of mature osteoclasts.[8] TNF-α is secreted in large quantities by osteolytic breast cancer cell lines and by breast tumors in bone. We have used a mouse model of breast cancer osteolysis to examine the timing of TNF-α expression as it relates to the appearance of osteolytic lesions. TNF-α expression by mouse marrow cells increases as tumor size increases and as osteolytic lesions appear in the mice. We have examined the effects of TNF-α added to osteoclast precursors during differentiation. TNF-α treatment results in increased osteoclast numbers and larger osteoclasts as compared to untreated osteoclasts. These larger TNF-α-stimulated osteoclasts secrete more bone-degrading enzymes and form more resorption pits per cell than unstimulated osteoclasts. TNF-α also appears to prolong the lifespan of mature osteoclasts by inhibiting apoptosis.[9] These results indicate that elevated TNF-α levels may play a role in metastatic breast cancer-induced osteolysis. TNF-α secreted by tumor cells and marrow cells may induce formation of larger osteoclasts that destroy bone at a rate faster than it can be replaced by osteoblasts.

Insulin-Like Growth Factors

Insulin-like growth factors (IGFs) are secreted by osteoblasts and stimulate proliferation of osteoblasts. Mature osteoclasts express type I IGF receptors and IGF stimulates resorption activity of mature osteoclasts in the presence of osteoblasts.[10] IGF induction is responsible for growth hormone and parathyroid hormone-induced osteoclast formation.[11] This suggests that IGF may contribute to osteoclast formation indirectly by inducing expression of another osteoclast-promoting growth factor. IGF is secreted by breast cancer cell lines and metastatic breast tumors in bone. IGF treatment of osteoclast precursors induces differentiation into mature osteoclasts. IGF secreted by metastatic breast tumors may contribute to elevated osteoclast activity by inducing differentiation of osteoclast precursors and stimulating resorption activity of mature osteoclasts.

Parathyroid Hormone-Related Peptide Expression

Parathyroid hormone-related peptide (PTHrP) expression is correlated with increased metastasis of breast cancer cells, which leads to increased frequency of osteolytic lesions in vivo. PTHrP is expressed by mature osteoclasts and 92% of breast tumors showing bone metastases.[12] PTHrP is the main causative agent of hypercalcemia that is associated with a variety of cancers. Hypercalcemia is mediated through an endocrine mechanism involving PTH receptor-mediated effects on kidney and bone metabolism. Paracrine actions of PTHrP are dual, with different portions of the PTHrP molecule giving rise to opposing effects. PTHrP(1-34) mediates stimulatory PTH-like actions by binding to the PTH receptor expressed on osteoblasts. These indirect effects include stimulation of osteoclast formation and increased bone resorption activity. However, PTHrP(107-139) mediates inhibitory actions of PTHrP, including direct inhibition of osteoclastic bone resorption, by an unknown PTH receptor-independent mechanism.[13] PTHrP expression appears to be permissive for formation of bone metastases, although its role in the induction of osteolysis remains unclear.

Interleukins

Interleukin-1 (IL-1) stimulates bone resorption primarily by prolonging survival of mature osteoclasts. IL-6 also stimulates bone resorption, but its mode of action is stimulation of osteoclast formation. IL-6 induces proliferation of osteoclast precursors and induces these precursors to commit to the osteoclast lineage. Infusion of IL-1 together with IL-6 in mice causes marked bone loss that results in hypercalcemia. Bone loss due to elevated levels of parathyroid hormone or 1, 25-dihydroxyvitamin D3 is due to the ability of these hormones to induce IL-6 secretion from osteoblasts.[6] Many breast tumors express IL-1 and IL-6, which makes these factors possible mediators of bone loss associated with metastatic breast cancer.

Leukemia Inhibitory Factor

Leukemia inhibitory factor (LIF) is so named because of its ability to inhibit proliferation and induce differentiation to the macrophage line of a myeloid leukemic cell line.[14] Since its discovery, a variety of systemic effects have been attributed to LIF. The overall effect of LIF on bone metabolism is to increase the rate of bone turnover by increasing both osteoblast and osteoclast activity. LIF overexpression in mice causes splenic enlargement due to the excessive proliferation of hematopoietic stem cells, the cells that give rise to osteoclasts. LIF prolongs survival of osteoclast precursors and induces the proliferation of osteoblasts. LIF expression is induced by cytokines known to induce bone loss, including TNF-α, IL-1, and IL-6.[15] LIF is expressed by 78% of primary breast tumors and stimulates the proliferation of breast cancer cell lines in vitro.[16] Expression of LIF by breast cancers in bone could potentially stimulate osteoclast activity to induce bone loss.

Transforming Growth Factor

Transforming growth factor (TGF-β) has biphasic effects on osteoclast activity. At high doses, TGF-β inhibits osteoclast formation. However, at low doses, TGF-β stimulates formation and survival of osteoclasts. We have shown that osteoclasts formed in the presence of TGF-β become TGF-β-dependent. Withdrawal of TGF-β from these TGF-β-dependent osteoclasts induces immediate apoptosis.[17] Osteoclasts formed in the environment adjacent to a TGF-β-secreting breast tumor may become TGF-β-dependent and may survive longer than osteoclasts formed in the absence of TGF-β. Enhanced survival of TGF-β-dependent osteoclasts may provide a mechanism by which breast tumors can induce osteolysis.

Summary

Breast tumors in the bone marrow cavity can induce bone loss by inducing the formation, activity, and survival of mature osteoclasts. These effects may be due to the secretion of growth factors in the area adjacent to the tumor. RANKL, M-CSF, TNF-α, IGF, IL-1, IL-6, LIF, and TGF-β can enhance formation of mature osteoclasts by exerting effects on various stages of osteoclast differentiation. RANKL, M-CSF, TNF-α, IGF, and LIF can promote osteoclast resorption activity by increasing adhesion to bone, secretion of lysosomal proteases, and migration. M-CSF, TNF-α, IL-1, and TGF-β increase the lifespan of mature osteoclasts by delaying apoptosis. Additionally, repression of factors that block osteoclast formation, such as GM-CSF, may enable tumors to recruit osteoclasts. In order to clarify the role of tumor-derived growth factors in development of osteolytic lesions, future investigations should explore the combined effects of growth factors on osteoclast activity. *(Ms. Shaw is a research assistant and Dr. Oursler is an Assistant Professor, Biology Department, University of Minnesota, Duluth.)* ❖

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Polyamines and Polyamine Regulators in Prostate Cancer

By David A. Corral, MD

The steps to progression for any given tumor are under the control of complex regulatory events, with the overall growth rate determined by the balance of the number of cells undergoing proliferation, cell death, and quiescence. Among the many factors that influence these events are polyamines, the most basic (positively charged) small organic molecules that are found in high concentrations in several tissues and affect diverse physiologic processes that influence cell proliferation and growth. Increases in the level of the aliphatic polyamines putrescine, spermidine (SPD), and spermine (SPM), which are necessary for normal and pathological cell growth, have been associated with proliferation and transformation induced by growth factors, carcinogens, viruses, and oncogenes.[1,2] Polyamine metabolism is under complex regulation by multiple enzymes. In the prostate, polyamines are present in high concentration, and levels are positively controlled by androgens. Multiple attempts have been made at correlation of individual polyamine levels with tumor growth and progression, but because of the complex nature of the effects of these molecules, no single polyamine has proved to be a useful molecular marker of tumor progression.[1]

Expression of Polyamine Metabolism Regulatory Genes

Recently, Saverio et al published a report on the expression of various polyamine metabolism regulatory genes that encode enzymes that control polyamine synthesis and degradation in prostate cancer specimens and attempted to correlate these findings with clinical grade and stage.[3] A total of 23 prostatectomy specimens were dissected to obtain 0.5 cc tumor specimens and control tissue from adjacent, normal areas of the gland.

It should be noted that each patient in this study had received three months of neoadjuvant hormone ablation therapy prior to surgery. The authors used Northern blot analysis on RNA obtained from the specimens to examine relative levels of expression of the following genes: 1) ornithine decarboxylase (ODC), the rate-limiting enzyme of polyamine synthesis and a putative proto-oncogene;[4] 2) ornithine decarboxylase antizyme (OAZ), which inhibits ODC activity by accelerating its degradation and is induced by high levels of intracellular polyamines; 3) adenosylmethionine decarboxylase (AdoMetDC), also a rate-limiting enzyme of polyamine synthesis; 4) spermidine/spermine N1-acetyltransferase (SSAT), an enzyme involved in polyamine degradation and excretion; and 5) clusterin, also known as sulfated glycoprotein 2, a heterodimeric glycoprotein upregulated during proliferation but downregulated during atrophy, such as in the prostate following androgen ablation. For comparison, the authors also determined levels of expression of histone H3, a marker of cell proliferation, and Gas1, which is involved in growth suppression and maintenance of the quiescent state.

Prostate cancer is graded according to the Gleason grading system, where a score is assigned to the two most predominant patterns seen in the tumor. These are added together to obtain the Gleason score for the tumor, which ranges from a low of 2 (lowest grade, least aggressive) to a high of 10 (most aggressive). Saverio et al compared expression of the polyamine metabolism regulator genes described above to the grade of each tumor. Not surprisingly, low grade Gleason score 2 tumors had increased levels of Gas1 but decreased levels of H3 mRNA relative to controls, whereas in Gleason score 5 or 8 tumors Gas1 was downregulated and H3 was overexpressed.

With regard to the expression of the regulatory proteins of polyamine metabolism, the authors demonstrated that in low-grade prostate cancer specimens (assumed low proliferative activity), ODC is upregulated with a concomitant increase in the expression of OAZ. Expression of AdoMetDC was unchanged and only a minor increase in SSAT mRNA was detected.

Saverio et al conclude that these findings are consistent with the hypothesis that ODC induction, which should be balanced by activation of the regulatory steps of polyamine metabolism to prevent polyamine overaccumulation, is an early event during cell transformation. In higher grade Gleason 8 cancers (presumed more actively proliferating), induction of ODC was not counterbalanced by OAZ overexpression. AdoMetDC mRNA was dramatically elevated in the high grade specimen, and the increased levels of the polyamines SPD and SPM, which would follow the induction of the two biosynthetic enzymes, may have been partially balanced by SSAT overexpression. The authors propose that this constellation of findings would result in intracellular polyamine concentrations capable of supporting a high rate of cell proliferation without jeopardizing cell survival. Clusterin was downregulated in both Gleason grade 2 and grade 8 tumors, which the authors interpreted as evidence of clusterin downregulation being an early event of prostate tumor development. The trends

detected at the mRNA level were confirmed at the protein level by Western blot analysis for clusterin and by enzymatic assays for SSAT and AdoMetDC. The authors also detected a trend toward increased histone H3 mRNA levels and decreased Gas1 mRNA relative to normal controls, but this was not statistically significant.

Saverio et al went on to correlate their findings with clinical prognostic indicators. Tumors from patients with negative prognostic indicators, such as elevated prostate specific antigen (PSA) following surgery, lymph node involvement, or the presence of distant metastases, demonstrated significant upregulation of ODC, AdoMetDC, and SSAT, whereas Gas 1 and SGP-2 were downregulated. SSAT was also overexpressed in patients with a favorable prognosis.

An Alternative Tumor Classification Scheme?

Similar findings were noted when patients were stratified by PSA level. Using logistic regression to evaluate the predictive ability of changes in expression of the entire panel of the genes studied, the authors sought to establish an alternative tumor classification system. Changes in *H3* and *Gas 1*gene expression were predictive of tumor localization (i.e., organ confined vs capsular penetration) in 72% of cases, and the accuracy was raised to 83% when all genes were included. Perhaps more importantly, two patients who were placed in the poorly differentiaited group by virtue of the results of their polyamine regulatory gene profiles but who had a moderate Gleason histologic score have had a rising PSA within one year of radical surgery, indicating cancer recurrence.

Summary

The article by Saverio et al points out the complexities of polyamine biology and its involvement in the regulation of the cell cycle. The authors elegantly examined the relationship of the various regulatory molecules in varying grades of prostate cancer. In their report, the authors do, however, make the assumption that there is a true progression from Gleason grade 2 prostate cancer through to grade 10 cancer as the disease progresses. While it is unquestionably true that the higher grade cancers behave more aggressively, it is not so clear that prostate cancer begins as a low-grade tumor and progressively de-differentiates in a step-wise fashion to a poorly differentiated state.

The fact that latent low-grade prostate cancer is a frequent incidental finding at autopsy and would likely never have become apparent, whereas clinically significant cancer presents at higher grade and stage, would argue against such a regimented, step-wise progression. Nevertheless, Saverio's finding of differences among differing grades is certainly important. While the biology surrounding polyamine action, synthesis, and degradation is certainly interesting, the question arises of how therapeutically useful this information is and how it could be specifically applied in the clinical setting.

The data from Saverio's study are interesting in that they demonstrate that a molecular profile of the expression of the genes involved in polyamine metabolism may provide information that the current Gleason grading system and clinical preoperative staging cannot. If such a classification scheme could predict, at the time of diagnosis, who is likely to have a cancer recurrence following surgery, then therapy could be directed more appropriately toward other modalities, thus sparing the patient the potential associated morbidities. Of course, the utility of such a profile would need to be tested in a large scale prospective fashion. ❖

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Role of MIP1α in Anti-Tumor Immunity and Therapy

By Maja Maric, PhD

Specific immune responses are usually preceded by local inflammation. During an anti-tumor immune response, T cells and antigen presenting cells (APCs), in addition to other leukocytes, are recruited into the tumor. It is clear that T cell effector response requires inflammation, which brings leukocytes into contact with tumor cells. What causes the selective leukocyte recruitment into solid tumor? The molecular basis for this selective recruitment in tumor is still being explored, but a family of proteins called chemokines are emerging as major players in these processes. Therefore, our ability to manipulate the recruitment of a specific

subset of leukocytes into tumor may enhance the anti-tumor immune response.

It is widely accepted that the most important effector cells in tumor immunity are activated T cells, especially CD8+ subset, because most somatic tissues express MHC class I but not class II molecules. However, their arrival into the tumor is usually preceded by cells of innate immunity like macrophages, neutrophils, and natural killer (NK) cells, which also have the ability to kill tumor cells. How do all these immune cell subsets find their way to a site on the tumor in an ordered sequence? Our immune system is equipped with a "911 system" that brings all potential help to the area that needs the help most. A very important part of this "911 system" is made of a family of proteins called chemokines. Among the four known families, a-, b-, g-, and d-family, potentially the most interesting family for tumor immunotherapy is b-family, whose members show ability to selectively attract monocytes, eosinophils, and lymphocytes. One of the most explored and interesting members of this group is macrophage inflammatory protein 1α (MIP1α), known also as LD78. MIP1α is involved in inducing chemotaxis and inflammatory responses, and in the homeostatic control of stem cell proliferation. It is produced by a variety of cells: macrophages, neutrophils, T cells (preferentially CD8+ subset), fibroblasts, endothelial cells, B cells, and NK cells.[1,2] MIP1α acts through interaction with its receptor, CCR5 (also a co-receptor for HIV entry). This chemokine has been detected at locally increased levels in various pathological states from injuries to autoimmune disorders like rheumatoid arthritis, tumors, infection with intracellular parasites, or viruses.[3-6]

Background

It has been shown that MIP1α can selectively recruit CD8+ T cells and macrophages, and that MIP1α-/- mice have significantly reduced inflammatory response to influenza virus infection.[7-9] In vivo studies using mouse plasmacytoma with the defined tumor antigen (P1A) as tumor model have shown that local expression of MIP1α results in strong inflammation of leukocytes in tumors and leads also to the induction of strong anti-tumor immune response.[10]

When plasmacytoma was manipulated to express different levels of MIP1α, increased infiltration of APCs (macrophages, B cells, and dendritic cells) was detected without alteration of their composition in infiltrate. When tumor-infiltrating leukocytes (TILs) were isolated from MIP1α secreting tumor and used in cytotoxic assay, TILs from MIP1α secreting tumor were 3- to 30-fold more efficient in lysis of target cells than TILs from the tumor that did not express MIP1α. However, despite the drastic difference in CTL response there was no difference in the rate of tumor growth between tumors secreting MIP1α and tumors that were not secreting MIP1α. This result suggests that the production of cytotoxic lymphocytes within tumor is not sufficient to cause tumor rejection. In ex vivo CTL assay targets expressing costimulatory molecule B7 were more efficiently lysed than targets without costimulatory molecule. This leads us to the question of whether costimulatory molecules B7-1 and B7-2, expressed by host APCs, can play a role in the induction of CTLs.

In the same study, two groups of mice were injected with MIP1α-secreting tumors and treated with either PBS or anti-B7-1 and anti-B7-2 monoclonal antibodies, and cytotoxic activity was measured at different time points. In mice treated with anti-B7 antibodies, tumor antigen-specific cytotoxic activity was significantly abrogated while the control group developed significant cytotoxicity. These experiments demonstrate that local/tumor expression of MIP1α can induce strong CTL response against tumor antigen without further in vitro restimulation. The fact that in vivo tumors are not rejected could be explained by the requirement for B7 costimulatory molecules directly on tumor cells and/or still undefined tumor factors (related or unrelated to MIP1α) with ability to suppress anti-tumor CTL activity. This is plausible, because tumor antigens are, in most cases, self-antigens and self-reactive T cells would be potentially harmful for host organisms. If both antigen and costimulatory molecules are required at the effector phase of T cells, it suggests that tumor CTLs are not fully activated.

Cell Cycle Arrest and MIPIa

Another potentially useful function of MIP1α in tumor immunotherapy at a more systemic level is its ability induce cell cycle arrest in immature hematopoietic progenitors, and it could, therefore, be used to reduce the hematologic toxicity of cell cycle active therapy. Recently, a genetically engineered analog of MIP1α, BB-10010, has been used in several studies on mice and in clinical trial.[11-13] This variant of MIP1α has single amino acid substitution of Asp26>Ala that confers a reduced tendency of this molecule to form large, less active polymers at physiologic pH and ionic strength.[14] This variant of MIP1α has been tested in several studies for potential protective effects in chemotherapy-induced neutropenia, which is a major dose-limiting factor in chemotherapy. Most chemotherapeutic reagents are active against proliferating cells by interfering with DNA replication and mitosis. BB-10010 shows potential protective abilities because it reduces accumulated hematopoietic stem cell damage following repeated non-cell cycle specific cytotoxic insults.[11] In another study, it

also reduced toxicity of three different cytotoxic drugs: cyclophosphamide, 5-fluorouracil, and cytosine arabinoside.[12] In a phase I study with cancer patients and healthy volunteers, doses of BB-10010 from 10-300 mg/kg (given IV) were well tolerated, although it caused acute, short-lived monocytopenia.[13] It remains to be seen whether further clinical studies will confirm usefulness of MIP1α variant BB-10010 in chemotherapy.

Summary

Animal and human studies with MIP1α suggest that this chemokine and/or its analog BB-10010 may have two important roles in the fight against cancer. When expressed locally in tumor tissue, it attracts effector cells and acts as an additional costimulatory factor in activation of CD8+ T cells. Also, when distributed systemically it may protect hematopoietic stem cells from damage from chemotherapy. The whole picture is more complicated because in animal studies described here, the sole action of MIP1α is not sufficient to induce such a vigorous cytotoxic response in vivo that it would be expected to cause immediate tumor rejection. Rather, when working in conjunction with other costimulatory molecules like B7, its action is more effective. It should also be kept in mind that chemokines in general bind promiscuously to a family of receptors and that such complicated interplay of many factors is easy to throw out of balance and difficult to control. By focusing on one chemokine, we cannot exclude the possible roles of others that bind and compete for the same receptor.

Further studies are necessary to explore more details and additional factors that may alter or control the role of MIP1α as a locally expressed factor in the tumor immunotherapy. As a protective agent from negative effects of chemotherapy, BB-10010 is certainly promising, and perhaps more agents like that should be defined while the scientific community searches for less toxic ways of fighting the cancer. *(Dr. Maric is a Post-Doctoral Fellow, Immunobiology Section, Yale School of Medicine, New Haven, CT.)* ❖

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Funding News

The Kidney Cancer Association has announced plans to fund three multi-year grants for research into the causes and treatments of kidney cancer totaling $450,000. The Association has recently received the largest grant in its l0-year history in the amount of $270,842 from the Falk Medical Research Trust. The grant money will be used to fund a Senior Career Investigator Award. Funds from other sources will also be

used to support the annual Eugene P. Schonfeld Medical Research Award, and the David A. Seulberg Career Investigator Award.

Senior Career Investigator Award

The Senior Career Investigator Award provides salary support or project support for a senior physician or scientist investigator working on the prevention and/or treatment of kidney cancer, and amounts to a total of $250,000. For the first year, $80,000 is provided, with an option for a second and third year at $85,000 each, based on productivity. The award is intended for a senior individual, one who has devoted at least l0 years to the field of kidney cancer research. It is expected that a Senior Career Investigator Award recipient will devote 25% of his effort to the project described in the award. The awardee may also undertake limited administrative, teaching, and clinical responsibilities if they are directly related to the nature of the research being supervised. Funding will be based on experience, future plans, publications, letters of recommendation, and potential for making a significant contribution to kidney cancer research. Proposals should be submitted in the National Institutes of Health (NIH) format, nine pages or less, including references. Funding is provided for direct costs only. The use of funds and other support should be described in the application. Ten copies of the proposal, along with a curriculum vitae and three letters of recommendation, should be submitted to the Kidney Cancer Association.

Eugene P. Schonfeld Medical Research Award

The Kidney Cancer Association is also offering the Eugene P. Schonfeld Medical Research Award, which provides $l00,000 over a three-year period to a young MD, DO, or PhD working in the field of kidney cancer research. This proposal should also be made in the NIH format, nine pages or less, including references. Funding through the Eugene P. Schonfeld Medical Research Award is provided for direct costs only and the use of funds should be described within the proposal. Awards are made payable over a two-year period in equal payments of $50,000 each. The award is intended for candidates who are not more than five years out from their post-doctoral degree. The application should focus on the theory, originality, technical approach, and potential for new contributions of the proposed project. Applications should be sent to the Kidney Cancer Association at the address below.

David A. Seulberg Career Investigator Award

The David A. Seulberg Career Investigator Award offered by the Kidney Cancer Association provides $35,000 per year for two years, with an option for an extension to a third year based on productivity. The money is provided as salary and/or project support for an MD, DO, or PhD working on the prevention and/or treatment of kidney cancer. The grant is intended for an individual who is making the transition from junior to mid-level faculty, ideally an investigator with between five and 10 years of experience. It is expected that the investigator will devote 30% of his effort to the project described in the award. This award is not available to individuals who are either full professors or who have less than five years or more than l0 years of faculty experience. The application should also be placed in the NIH format, nine pages or less, including references. The application, along with curriculum vitae and three letters of recommendation, should be submitted to the Kidney Cancer Association at: Kidney Cancer Association, 1234 Sherman Ave., Suite 203, Evanston, IL 60202-l375. Phone: (847)-332-1051. Web site: www.nkca.org ❖

CME Questions

14. The most important effector cells in tumor immunity are:
- a. CD4+ B cells.
- b. CD4 - B Cells.
- c. CD8 + T Cells.
- d. CD8 - T Cells.

15. Women with lung cancer are more likely:
- a. to be diagnosed at an early age than men.
- b. more likely to be lifetime non-smokers.
- c. likely to smoke fewer cigarettes per day than males with lung cancer.
- d. all of the above.

16. Antibody blockade of TNF-* results in:
- a. decreased osteoclast numbers.
- b. increased pit formation activity of mature osteoclasts.
- c. decreased osteoblast numbers.
- d. decreased chondroblast numbers.

17. Ornithine decarboxylase:
- a. is activated by ornithine decarboxylase antizyme.
- b. is the rate-limiting enzyme of polyamine biosynthesis.
- c. catabolizes clusterin.
- d. is a polyamine.

18. Alipathic polyamines include all of the following *except*:
- A. putrescine.
- B. scopalamine.
- C. spermidine.
- D. spermine.

In Future Issues: CpG Island Methylation