CXCL12 and CXCR4 in adenocarcinoma of the lung: Association with metastasis and survival

Patrick L. Wagner, MD,^a Elizabeth Hyjek, MD,^b Madeline F. Vazquez, MD,^a Danish Meherally, MPH,^c Yi Fang Liu, MD,^a Paul A. Chadwick, BS,^a Tatiana Rengifo, BS,^a Gabriel L. Sica, MD,^a Jeffrey L. Port, MD,^c Paul C. Lee, MD,^c Subroto Paul, MD,^c Nasser K. Altorki, MD,^c and Anjali Saqi, MD^a

Objectives: Although the chemokine CXCL12 and its receptor CXCR4 have been implicated in metastasis of non–small cell lung carcinoma, the prognostic significance of these molecules is poorly defined. This study aimed to determine whether expression of these molecules is associated with clinicopathologic features and disease-free survival in non–small cell lung carcinoma.

Methods: Immunohistochemical staining for CXCL12 and CXCR4 was performed on 154 primary non–small cell lung carcinomas. Staining intensity was compared with tumor histotype, TNM stage, and disease-free survival; correlation was assessed by using the Fisher's exact test, and Kaplan–Meier and Cox multivariate proportional hazards regression analysis.

Results: Intense CXCL12 immunostaining was associated with nodal metastasis, although no difference in survival was observed. The prognostic relevance of CXCR4 was dependent on its subcellular location: in univariate analysis intense nuclear staining was significantly associated with lower T classification and improved disease-free survival in patients with adenocarcinoma, whereas cytomembranous staining was associated with distant metastasis and decreased disease-free survival. On multivariate analysis, cytomembranous CXCR4 expression conferred a significantly worse disease-free survival (relative risk, 2.8; 95% confidence interval, 1.4–5.7; $P = .004$).

Conclusions: Cytomembranous expression of the chemokine receptor CXCR4 in adenocarcinoma of the lung is an independent risk factor associated with worse disease-free survival, whereas nuclear staining confers a survival benefit. These findings are consistent with a model in which CXCR4 promotes tumor cell proliferation and metastasis when present in the cytoplasm or cell membrane, whereas localization of this molecule in the nucleus prevents it from exerting these effects.

Chemokine receptors constitute a family of 7-transmembrane proteins that interact specifically with chemokines, a subset of cytokines known for their role in cell movement along a chemical gradient (chemotaxis). On exposure to their specific chemokine ligands, the receptors are internalized within endosomes and participate in signal transduction, culminating in cytoskeletal rearrangement and directional migration toward the chemokine source. This process is vital in many physiologic events, including migration of precursor cells during development and homing of leukocytes to injured or infected tissues. In addition to their involvement in physiologic processes, these molecules play an increasingly understood role in the progression of a large number of human malignancies by stimulating the proliferation, invasion, and metastasis of tumor cells.^{1–3}

Non–small cell lung carcinoma (NSCLC) cells have been shown to express the chemokine receptor CXCR4. These cells undergo increased proliferation, chemotaxis, and metastasis in vitro and in murine models in response to CXCL12, the specific chemokine ligand for CXCR4.³ In the lung CXCL12 is produced by pulmonary epithelial cells and normally functions to recruit leukocytes to the airspaces during acute lung injury.⁴ Because malignant lung tissue has the capacity to express both CXCL12 and its receptor, CXCR4, it is hypothesized that these molecules could drive tumor progression by means of autocrine or paracrine mechanisms. However, although experiments in murine models have yielded results consistent with this model, the clinical relevance of chemokine receptor expression in lung cancer and other common epithelial malignancies remains uncertain.

The aim of this study was to quantitate the expression of CXCL12 and CXCR4 in a series of primary NSCLCs and to determine whether these molecules are associated with clinicopathologic features of NSCLC, including tumor histologic type, multifocality, staging parameters, and disease-free survival (DFS).

MATERIALS AND METHODS

Case Selection

We retrospectively selected 154 primary NSCLCs from 121 patients undergoing resection with curative intent in a single institution during

From the Department of Pathology and Laboratory Medicine^a and Cardiothoracic Surgery,^c New York Presbyterian Hospital/Weill Cornell Medical Center, New York, NY, and the Department of Pathology,^b University of Chicago, Chicago, Ill. Received for publication April 4, 2008; revisions received July 1, 2008; accepted for publication July 26, 2008.

Address for reprints: Patrick L. Wagner, MD, Department of Pathology and Laboratory Medicine, New York-Presbyterian Hospital/Weill Cornell Medical Center, Starr-1036, 525 E 68th St, New York, NY 10021 (E-mail: plw9001@nyp.org).

J Thorac Cardiovasc Surg 2009;137:615–21

0022-5223/\$36.00

Copyright © 2009 by The American Association for Thoracic Surgery

doi:10.1016/j.jtcvs.2008.07.039

Abbreviations and Acronyms

BAC	= bronchioloalveolar carcinoma
ERK	= extracellular signal-regulated kinase
NSCLC	= non-small cell lung carcinoma
VHL	= von Hippel-Lindau

a 12-year period. Cases were selected to ensure adequate representation of histologic subtypes and stages. Hematoxylin and eosin-stained slides from each primary lesion were reviewed, and the tumors were classified according to World Health Organization guidelines.⁵ Staging was performed according to the American Joint Committee on Cancer TNM system.⁶ The clinicopathologic features are summarized in Tables 1 and 2. Lesions were categorized as squamous cell carcinoma (n = 20), adenocarcinoma (n = 132), or adenosquamous carcinoma (n = 2); the adenocarcinomas were further subclassified as bronchioloalveolar carcinoma (BAC) subtype only (n = 38), patterns other than BAC (ie, acinar, papillary, and solid patterns; n = 18), or mixtures of BAC and non-BAC adenocarcinoma (n = 76). Of the 154 tumors, 87 were unifocal, and the remaining 67 tumors were resected from 33 patients who presented with more than 1 lesion. These 67 multifocal lesions, which theoretically include both synchronous primary metastases, as well as intralobar satellites and pulmonary metastases, were staged in accordance with American Joint Committee on Cancer guidelines: multifocal lesions with identical histologic features were staged as T4 if present within the same lobe and M1 if present within different lobes; lesions with different histologic features or without invasion (ie, pure BAC) were considered synchronous primary carcinomas and staged independently.⁶

Clinical features and outcome data were obtained from a prospectively established thoracic surgery database. Follow-up information, including evidence of recurrence in history and physical examination findings and in radiology and pathology reports, was obtained from office notes and hospital records. Median length of follow-up for adenocarcinomas was 2.2 years (range, 3 months to 15 years). Institutional review board permission for the study was obtained, and patient consent was waived in accordance with the Health Insurance Portability and Accountability Act.

Immunohistochemistry

Immunohistochemical staining was performed on 5- μ m-thick, formalin-fixed, paraffin-embedded tissue sections by using the BondMax Automated Immunohistochemistry and In Situ Hybridization System (Leica-Vision Biosystems, Melbourne, Australia) and the Bond Polymer Define peroxidase detection system (Leica-Vision Biosystems). Anti-CXCL12 antibody (clone 79018; R&D Systems, Minneapolis, Minn) and anti-CXCR4 antibody (clone 12G5, R&D Systems) were used at dilutions of 1:250 and 1:150, respectively, in Bond Primary Antibody Diluent (Leica-Vision Biosystems) with 0.5% casein block after antigen retrieval with Bond Epitope Retrieval Solution no. 1 for 30 minutes. Immunostaining was performed according to a modified manufacturer's protocol: incubation with the primary antibody (25 minutes), a postprimary step (15 minutes), and a detection step (Bond Polymer Define system, 25 minutes). The peroxidase reaction was developed with diaminobenzidine. Sections were counterstained with hematoxylin, dehydrated, and embedded in Cytoseal TMSXL (Richard Allan Scientific, Kalamazoo, Mich). Appropriate negative controls lacking primary antibody were performed.

CXCL12 staining was observed in the cytoplasm and cell membranes of NSCLC cells but not in the nuclei. CXCR4 staining was observed in both a nuclear and cytomembranous pattern in malignant cells, and intensity for these 2 compartments was scored independently. Intensity was scored as 0+ (absent), 1+ (low intensity), 2+ (intermediate), 3+ (high intensity), or

TABLE 1. Association of CXCL12 immunohistochemical staining with clinicopathologic features

Clinicopathologic feature	Overall (n = 150)	CXCL12 IHC intensity		P value
		Strong (n = 47)	Weak-absent (n = 103)	
Mean age (y)	67 $\pm$ 8	66 $\pm$ 8	67 $\pm$ 8	.6*
Sex				
Male	44	19 (43)	25 (57)	.05†
Female	106	28 (26)	78 (74)	
Histology				
Adenocarcinoma-mixed subtype‡‡	75	22 (29)	53 (71)	
Adenocarcinoma-BAC only‡‡	37	7 (19)	30 (81)	.4‡
Adenocarcinoma-non-BAC‡‡	18	3 (17)	15 (83)	
*Adenocarcinoma-total	130	32 (25)	98 (75)	.0001§
Squamous	18	14 (78)	4 (22)	
Adenosquamous	2	1	1	
Focality				
Unifocal	84	33 (39)	51 (61)	.02
Multifocal	66	14 (21)	52 (79)	
T score				
T1	78	18 (23)	60 (77)	.03¶
T2	45	21 (47)	24 (53)	
T3	1	1	0	
T4	26	7 (27)	19 (73)	
N score				
N0	108	27 (25)	81 (75)	.01#
N>0	42	20 (48)	22 (52)	
M score				
M0	144	44 (31)	100 (69)	.5**
M1	6	3 (50)	3 (50)	
Stage				
I	89	20 (22)	69 (78)	.007††
II	17	9 (53)	8 (47)	
III	38	15 (39)	23 (61)	
IV	6	3 (50)	3 (50)	

Percentages are shown in parentheses. IHC, Immunohistochemistry; BAC, bronchioloalveolar carcinoma. *P value corresponding to the Student *t* test comparing age among strong staining versus weak-absent staining for each antibody. The remaining P values correspond to the Fisher's exact or χ^2 test comparing strong versus absent staining for each antibody among subgroups defined as follows: †male versus female; ‡mixed versus BAC only versus non-BAC adenocarcinoma subtypes; §adenocarcinoma (all subtypes) versus squamous carcinoma; ||unifocal versus multifocal; ¶T1 versus T2 to T4; #N0 versus N1 to N2; **M0 versus M1; ††stage I versus stage II to IV. ‡‡Histologic subtypes of adenocarcinoma, summed as "Adenocarcinoma-total" for comparison with other histologic types (squamous and adenosquamous).

4+ (very high intensity) individually by 2 pathologists (PLW and AS) who were blinded to staging and outcome information. The lesions were then jointly examined by both pathologists to resolve discrepancies in scoring; there was high concordance (>90%) in the initial interpretation of staining intensity. Tumors scoring 0+ or 1+ were categorized as having low-absent expression, whereas those designated as 2+, 3+ or 4+ were categorized as having high-level expression. In cases with intratumoral variability in staining intensity, the predominant pattern was recorded. Examples are provided in Figures 1 and 2.

The pathologist-assigned intensity scores were validated by using the Applied Imaging Ariol analysis system (Genetix Ltd, New Milton, United

TABLE 2. Correlation of CXCR4 immunohistochemical staining with clinicopathologic features

Clinicopathologic feature	Overall (n = 154)	Nuclear CXCR4 IHC			Cytoplasmic CXCR4 IHC		
		Strong (n = 62)	Weak-absent (n = 92)	P value	Strong (n = 47)	Weak-absent (n = 107)	P value
Mean age (y)	67 ± 8	67 ± 8	66 ± 8	.7*	66 ± 8	68 ± 9	.1*
Sex							
Male	46	17 (37)	29 (63)	.7†	15 (33)	31 (67)	.7†
Female	108	45 (42)	63 (58)		32 (30)	76 (70)	
Histology							
Adenocarcinoma–mixed subtype§§	76	30 (39)	46 (61)		29 (38)	47 (62)	
Adenocarcinoma–BAC only§§	38	23 (61)	15 (39)	.01‡	9 (24)	29 (76)	.2‡
Adenocarcinoma–non-BAC§§	18	4 (22)	14 (78)		4 (22)	14 (78)	
Adenocarcinoma–total	132	57 (43)	75 (57)	.01§	42 (32)	90 (68)	.1§
Squamous	20	3 (15)	17 (85)		3 (15)	17 (85)	
Adenosquamous	2	2	0		2	0	
Focality							
Unifocal	87	28 (32)	59 (68)	.02	21 (24)	66 (76)	0.06
Multifocal	67	34 (51)	33 (49)		26 (39)	41 (61)	
T score							
T1	80	40 (50)	40 (50)	.01¶	24 (30)	56 (70)	.4¶
T2	47	14 (30)	33 (60)		13 (28)	34 (72)	
T3	1	0	1		1	0	
T4	26	8 (31)	18 (69)		9 (35)	17 (65)	
N score							
N0	110	46 (42)	64 (58)	.6#	34 (31)	76 (69)	1.0#
N>0	44	16 (36)	28 (64)		13 (30)	31 (70)	
M score							
M0	148	58 (39)	90 (61)	0.2**	42 (28)	106 (72)	.01**
M1	6	4 (67)	2 (33)		5 (83)	1 (17)	
Stage							
I	90	40 (44)	50 (56)	.2‡‡	27 (30)	63 (70)	.03‡‡
II	19	5 (26)	14 (74)		4 (21)	15 (79)	
III	39	13 (33)	26 (67)		11 (28)	28 (72)	
IV	6	4 (67)	2 (33)		5 (83)	1 (17)	

Percentages are shown in parentheses. IHC, Immunohistochemistry; BAC, bronchioloalveolar carcinoma. *P value corresponding to the Student *t* test comparing age among strong staining versus weak-absent staining for each antibody. The remaining *P* values correspond to the Fisher's exact or χ^2 tests comparing strong versus absent staining for each antibody among subgroups defined as follows: †male versus female; ‡mixed versus BAC only versus non-BAC adenocarcinoma subtypes; §adenocarcinoma (all subtypes) versus squamous carcinoma; ||unifocal versus multifocal; ¶T1 versus T2 to T4; #N0 versus N1 to N2; **M0 versus M1; ††stage I versus stage II to IV; ‡‡stage I to III versus stage IV. §§Histologic subtypes of adenocarcinoma, summed as "Adenocarcinoma–total" for comparison with other histologic types (squamous and adenosquamous).

Kingdom).^{7,8} This high-throughput image analysis system was used to yield high-resolution numeric intensity scores for cytomembranous and nuclear staining with CXCR4 and CXCL12 on a tissue microarray constructed from the tumors analyzed in this study. The numeric scores, generated on a scale from 1 to 255, were tested for correlation with the pathologist-assigned intensity scores for each antibody on each tumor by using Spearman's rank coefficient test. Excellent overall agreement between the automated system and the pathologists' assessment was demonstrated for each antibody as follows: CXCL12 cytomembranous staining ($r_s = 0.5$, $P < .0001$), CXCR4 cytomembranous staining ($r_s = 0.21$, $P = .02$), CXCR4 nuclear staining ($r_s = 0.36$, $P = .0002$).

Statistical Analysis

The clinicopathologic and immunohistochemical features of the lesions were evaluated with either an unpaired Student *t* test or the Fisher's exact test, as appropriate. DFS curves were generated by using the Kaplan–Meier method (Prism 5; GraphPad Software, Inc, San Diego, Calif) and compared with the log-rank and Wilcoxon tests. A multivariate Cox proportional hazards regression model of independent prognostic factors for DFS was performed (SPSS, Inc, Chicago, Ill).

RESULTS

Association of CXCL12 Expression With Clinicopathologic Features and Outcome

CXCL12 immunostaining demonstrated a cytoplasmic and membranous distribution and was present to some extent in nearly every lesion, as well as in adjacent normal bronchial and alveolar epithelial cells. Sufficient material was available for CXCL12 immunostaining of 150 of the 154 primary NSCLC lesions (Figure 1 and Table 1), which were categorized as expressing high levels of CXCL12 (n = 47) or low-absent levels of CXCL12 (n = 103). Male patients tended to exhibit increased CXCL12 staining relative to female patients (43% vs 26%, $P = .05$). Squamous cell carcinomas (14/18 [78%]) were more likely to express high-level CXCL12 than adenocarcinomas (32/132 [25%], $P < .01$), whereas unifocal tumors (33/84 [39%]) more frequently expressed high-level CXCL12 than multifocal lesions

FIGURE 1. Immunohistochemical staining for CXCL12. A, Malignant cells exhibit low-absent CXCL12 reactivity compared with adjacent bronchial epithelium (*upper right*) and alveolar pneumocytes (*lower right*). B, Malignant cells in the *right half of the image* exhibit strong cytomembranous staining relative to the nonneoplastic pneumocytes in the *left half of the image*. C, Negative control lacking primary antibody; for comparison, the *inset* shows same area stained with CXCL12 primary antibody. Scale bars = 100 μ m.

(14/66 [21%], $P = .02$). Low-absent CXCL12 expression was associated with a lower T classification: 58% of these lesions were T1 compared with 38% of lesions expressing high levels of CXCL12 ($P = .02$). Likewise, node-negative lesions expressed lower levels of CXCL12 compared with tumors with positive lymph nodes (75% vs 52%, $P = .01$), and we observed a corresponding association of low-level CXCL12 expression with stage I disease (Table 1, $P = .007$). Despite the association of CXCL12 levels with differences in node status and stage, DFS did not significantly differ between lesions with high-level CXCL12 expression and those with low-level expression (Figure 3, D).

Association of CXCR4 Expression With Clinicopathologic Features and Outcome

Nuclear and cytomembranous CXCR4 immunostaining were scored separately (Figure 2) in all 154 primary

NSCLCs. High-intensity nuclear and cytomembranous staining was present in 62 (40%) of 154 and 47 (31%) of 154 lesions, respectively. Nuclear CXCR4 expression exhibited significant associations in univariate analysis with respect to histologic type of NSCLC, multifocality, T classification, and DFS (Table 2). Regarding histotype, squamous cell carcinomas were less likely than adenocarcinomas to express high-level nuclear CXCR4 (15% vs 43%, $P = .01$), whereas among subtypes of adenocarcinoma, lesions containing only BAC were more likely to express high-level nuclear CXCR4 than lesions exhibiting patterns other than BAC (61% vs 36%, $P = .01$). High-level nuclear staining was associated with multifocality: 51% of multifocal lesions showed high-level nuclear CXCR4 compared with 32% of unifocal lesions ($P = .02$). Lesions with high-level nuclear CXCR4 staining were more likely to be T1 than those with low-absent nuclear CXCR4 ($P = .01$). No significant

FIGURE 2. Immunohistochemical staining for CXCR4. A, Malignant cells in the *lower half of the image* exhibit low-absent nuclear and cytomembranous CXCR4 staining; note the strong staining of adjacent lymphocyte nuclei in the *upper portion of image*. B, Malignant cells in the *center of the panel* exhibit strong nuclear and low-absent cytomembranous staining; note interspersed CXCR4⁺ lymphocytes. C, Malignant cells exhibit strong nuclear and cytomembranous CXCR4 reactivity. D, Negative control lacking primary antibody; for comparison, the *inset* shows the same area stained with CXCR4 primary antibody. Scale bars = 100 μ m.

FIGURE 3. Disease-free survival (DFS) among adenocarcinomas with respect to CXCL12 and CXCR4 immunohistochemical staining. A, Association of cytomembranous CXCR4 with decreased DFS. B, Association of nuclear CXCR4 with increased DFS. C, Particularly favorable prognosis for lesions with the combination of high nuclear and low cytomembranous CXCR4 levels. D, Lack of significant association of CXCL12 with DFS. Numerals represent the number at risk at 1, 3, and 5 years.

association between nuclear CXCR4 expression and N or M classification was noted. High-level nuclear CXCR4 was associated with improved DFS relative to low-absent nuclear expression ($P = .02$; Figure 3, B).

Among the clinicopathologic features studied, cytomembranous CXCR4 staining was significantly associated only with distant metastases and DFS: M1 (stage IV) lesions were more likely to exhibit high-level cytomembranous CXCR4 staining than were M0 lesions (83% vs 28%, $P = .01$), and high-level cytomembranous CXCR4 expression was associated with a poorer prognosis among adenocarcinomas ($P = .04$; Figure 3, A).

As stated above, nuclear high-level CXCR4 expression was associated with a better prognosis, whereas cytomembranous CXCR4 expression was associated with a poorer prognosis. The combination of strong nuclear CXCR4 positivity and weak-absent cytomembranous staining was associated with a particularly favorable prognosis (hazard ratio, 0.38; 95% confidence interval [CI], 0.16–0.87; $P = .02$). To determine whether CXCR4 immunostaining carries independent prognostic implications, we conducted Cox multivariate proportional hazard ratio regression analysis. Univariate analysis identified a significant association of DFS with unifocality ($P = .02$), negative lymph node status ($P = .049$), high-level nuclear CXCR4 staining ($P = .047$),

and low-level cytomembranous CXCR4 staining ($P = .02$). In multivariate analysis incorporating staging parameters, nuclear CXCR4 staining was significantly associated with improved DFS, with a relative risk of death or recurrence of 0.38 (95% CI, 0.17–0.89, $P = .03$), whereas cytomembranous CXCR4 staining was significantly associated with decreased DFS (relative risk, 2.8; 95% CI, 1.4–5.7; $P = .004$), indicating that the observed effects are stage independent.

DISCUSSION

Chemokine receptors, in addition to their physiologic roles in development and immunity, play an increasingly recognized role in the progression of numerous human malignancies. Tumor cells expressing these receptors can respond to chemokines by undergoing increased proliferation, angiogenesis, and invasion. Moreover, because of the specific nature of the chemokine ligand–receptor interaction, metastasis could occur in a site-specific manner determined by the specific chemokine receptors expressed by circulating tumor cells and the subset of chemokines that are constitutively secreted by any given target organ.¹ The chemokine CXCL12 is expressed in common sites of metastasis, including lymph nodes, lungs, liver, and bone marrow, and in animal models the interaction between CXCL12 generated by

target organs and CXCR4 expressed on circulating breast and lung carcinoma cells has been demonstrated to be a vital step in the establishment of metastatic lesions in these sites.^{2,3} However, the clinical relevance of this interaction is poorly defined in NSCLC, and the purpose of this study was to assess whether CXCL12 and CXCR4 are associated with tumor progression and survival.

We found that these molecules are indeed associated with significant differences in clinicopathologic features among NSCLCs. On univariate analysis, we determined that CXCL12 expression is associated with nodal metastasis but has no measurable effect on DFS. CXCR4, when present in the nucleus, is associated with multifocality and lower T classification, whereas cytomembranous CXCR4 expression is linked with stage IV disease. Likewise, multivariate analysis revealed an independent association of CXCR4 with survival depending on its subcellular location, with nuclear staining correlating with favorable prognosis and cytomembranous staining correlating with poorer prognosis. Thus localization of CXCR4 within the nucleus of NSCLC cells appears to have an overall inhibitory effect on tumor progression, whereas cytomembranous CXCR4 is an independent risk factor for poor outcome.

CXCR4 expression in NSCLC has been established to play a role in tumor progression and metastasis in vitro and in murine models.^{3,9} Two studies have attempted to determine the clinical significance of these findings. Spano and colleagues,¹⁰ studying only stage I lesions, found an association between nuclear CXCR4 staining and increased survival among 61 patients. Because of their inclusion criteria, node-positive versus node-negative lesions could not be compared nor could T classification, multifocality, or stage. Su and associates⁹ observed high-level cytomembranous CXCR4 staining in 17 of 34 patients and noted a significant correlation between high cytomembranous CXCR4 staining intensity and metastasis in univariate analysis. However, interpretation of their data is restricted by the fact that lymph node, regional, and distant metastatic lesions were included as one group and important staging information was not provided, preventing assessment of the independent effects of CXCR4 expression on survival. By incorporating clinicopathologic features of 154 primary NSCLCs with outcome data in a multivariate analysis, our report is the first to establish CXCR4 expression as an independent prognostic indicator in NSCLC.

Regulation of CXCL12 and CXCR4 expression is incompletely understood. Both genes are negatively regulated by the von Hippel-Lindau (VHL) tumor suppressor protein, an effect that is removed under hypoxic conditions or because of inactivation of the *VHL* gene, which occurs frequently in NSCLC.^{11,12} Because loss of VHL leads to increased production of both receptor (CXCR4) and ligand (CXCL12) within the same cell, the result is an autocrine

stimulus for proliferation and metastasis, as previously described in NSCLC, hemangioblastoma, and renal cell carcinoma cell lines.^{13,14} Likewise, adjacent nonneoplastic bronchial and alveolar epithelial cells could also stimulate tumor progression in a paracrine fashion by secreting CXCL12.^{3,15} CXCR4 has also been shown to be upregulated in response to epidermal-derived growth factor in NSCLC and RET-derived oncogenes in papillary thyroid carcinoma, whereas epigenetic mechanisms are known to influence CXCL12 expression in carcinoma of the colon and breast.^{13,16-18} Further studies will be necessary to distinguish whether these mechanism, characterized in cell lines, are also important in upregulating CXCR4 and CXCL12 expression in vivo.

The downstream intracellular effects of stimulation of CXCR4 by CXCL12 have been well characterized. Binding of CXCL12 to CXCR4 results in receptor dimerization and endosomal internalization of the receptor-ligand complex. Once in the cell, CXCR4 maintains a close physical association with extracellular signal-regulated kinases (ERKs) and cyclophilin A in the vicinity of the nuclear membrane, where it participates in phosphorylation and nuclear translocation of ERK1/2, a downstream mediator of epidermal-derived growth factor signaling.¹⁹⁻²¹ The observation that nuclear localization of CXCR4 confers a prognostic benefit in NSCLC raises the possibility that CXCR4 might have an as-yet-undiscovered direct activity within the nucleus, perhaps related to ERK1/2 translocation, that inhibits tumor progression. Alternatively, Spano and colleagues¹⁰ proposed that sequestration of CXCR4 within the nucleus could prevent this receptor from exerting its tumor-promoting effects in the cytoplasm or cell membrane. Future experimentation will be required to address the mechanisms of nuclear localization and potential intranuclear activity of CXCR4. Interestingly, a similar association of nuclear CXCR4 expression with improved survival and cytomembranous expression with worse prognosis has recently been reported in breast carcinoma,²² suggesting the existence of a shared mechanism of CXCR4 activity in common epithelial malignancies.

Additional effects of the CXCL12-CXCR4 interaction in malignant cells include calcium mobilization and activation of Ras/Raf, mitogen-activated protein kinases, Phosphoinositide 3-Kinase (PI3), and Akt.^{3,19} Ultimately, stimulated cells undergo cytoskeletal rearrangement and altered transcription to assume a phenotype of increased proliferation, resistance to apoptosis, and enhanced migration and invasion of extracellular matrix.^{3,17} Presumably, the intracellular response of CXCR4-expressing tumor cells to CXCL12 accounts for the observed association of these molecules with indicators of tumor aggressiveness and progression, such as higher T classification, nodal and distant metastasis, and decreased DFS.

In summary, the chemokine receptor CXCR4 is associated with indicators of tumor progression and independently

predicts decreased DFS when present in the cytoplasm and cell membrane of NSCLC cells. Cytomembranous expression of CXCR4 could thus define a subset of patients with lung cancer who might benefit from small peptide inhibitors of CXCR4 currently under investigation.^{23,24} Further studies are necessary to discern the mechanisms and significance of nuclear localization of CXCR4, which appears to be associated with lower tumor stage and increased survival in lung and breast carcinoma.

References

1. Zlotnik A. Involvement of chemokine receptors in organ-specific metastasis. *Contrib Microbiol.* 2006;13:191-9.
2. Muller A, Homey B, Soto H, Ge N, Catron D, Buchanan ME, et al. Involvement of chemokine receptors in breast cancer metastasis. *Nature.* 2001;410:50-6.
3. Phillips RJ, Burdick MD, Lutz M, Belperio JA, Keane MP, Strieter RM. The stromal derived factor-1/CXCL12-CXC chemokine receptor 4 biological axis in non-small cell lung cancer metastases. *Am J Respir Crit Care Med.* 2003;167:1676-86.
4. Petty JM, Sueblinvong V, Lenox CC, Jones CC, Cosgrove GP, Cool CD, et al. Pulmonary stromal-derived factor-1 expression and effect on neutrophil recruitment during acute lung injury. *J Immunol.* 2007;178:8148-57.
5. Travis WD, Muller-Mermelink HK, Harris CC, eds. Pathology and genetics of tumours of the lung, thymus, and heart. Geneva: IARC Press; 2004.
6. Greene FL. American Joint Committee on Cancer. AJCC cancer staging atlas. New York: Springer; 2006.
7. Gokhale S, Rosen D, Sneige N, Diaz LK, Resetkova E, Sahin A, et al. Assessment of two automated imaging systems in evaluating estrogen receptor status in breast carcinoma. *Appl Immunohistochem Mol Morphol.* 2007;15:451-5.
8. Turbin DA, Leung S, Cheang MC, Kennecke HA, Montgomery KD, McKinney S, et al. Automated quantitative analysis of estrogen receptor expression in breast carcinoma does not differ from expert pathologist scoring: a tissue microarray study of 3,484 cases. *Breast Cancer Res Treat.* 2008;110:417-26.
9. Su L, Zhang J, Xu H, Wang Y, Chu Y, Liu R, et al. Differential expression of CXCR4 is associated with the metastatic potential of human non-small cell lung cancer cells. *Clin Cancer Res.* 2005;11:8273-80.
10. Spano JP, Andre F, Morat L, Sabatier L, Besse B, Combadiere C, et al. Chemokine receptor CXCR4 and early-stage non-small cell lung cancer: pattern of expression and correlation with outcome. *Ann Oncol.* 2004;15:613-7.
11. Miyakis S, Liloglou T, Kearney S, Xinarianos G, Spandidos DA, Field JK. Absence of mutations in the VHL gene but frequent loss of heterozygosity at 3p25-26 in non-small cell lung carcinomas. *Lung Cancer.* 2003;39:273-7.
12. Weir BA, Woo MS, Getz G, Perner S, Ding L, Beroukhi R, et al. Characterizing the cancer genome in lung adenocarcinoma. *Nature.* 2007;450:893-8.
13. Phillips RJ, Mestas J, Gharaee-Kermani M, Burdick MD, Sica A, Belperio JA, et al. Epidermal growth factor and hypoxia-induced expression of CXC chemokine receptor 4 on non-small cell lung cancer cells is regulated by the phosphatidylinositol 3-kinase/PTEN/AKT/mammalian target of rapamycin signaling pathway and activation of hypoxia inducible factor-1alpha. *J Biol Chem.* 2005;280:22473-81.
14. Zagzag D, Krishnamachary B, Yee H, Okuyama H, Chiriboga L, Ali MA, et al. Stromal cell-derived factor-1alpha and CXCR4 expression in hemangioblastoma and clear cell-renal cell carcinoma: von Hippel-Lindau loss-of-function induces expression of a ligand and its receptor. *Cancer Res.* 2005;65:6178-88.
15. Wald O, Izhar U, Amir G, Avniel S, Bar-Shavit Y, Wald H, et al. CD4+CXCR4highCD69+ T cells accumulate in lung adenocarcinoma. *J Immunol.* 2006;177:6983-90.
16. Borrello MG, Alberti L, Fischer A, Degl'innocenti D, Ferrario C, Gariboldi M, et al. Induction of a proinflammatory program in normal human thyrocytes by the RET/PTC1 oncogene. *Proc Natl Acad Sci U S A.* 2005;102:14825-30.
17. Castellone MD, Guarino V, De Falco V, Carlomagno F, Basolo F, Faviana P, et al. Functional expression of the CXCR4 chemokine receptor is induced by RET/PTC oncogenes and is a common event in human papillary thyroid carcinomas. *Oncogene.* 2004;23:5958-67.
18. Kubarek L, Jagodzinski PP. Epigenetic up-regulation of CXCR4 and CXCL12 expression by 17 beta-estradiol and tamoxifen is associated with formation of DNA methyltransferase 3B4 splice variant in Ishikawa endometrial adenocarcinoma cells. *FEBS Lett.* 2007;581:1441-8.
19. Pan H, Luo C, Li R, Qiao A, Zhang L, Mines M, et al. Cyclophilin A is required for CXCR4-mediated nuclear export of heterogeneous nuclear ribonucleoprotein A2, activation and nuclear translocation of ERK1/2, and chemotactic cell migration. *J Biol Chem.* 2008;283:623-37.
20. Zhao M, Discipio RG, Wimmer AG, Schraufstatter IU. Regulation of CXCR4-mediated nuclear translocation of extracellular signal-related kinases 1 and 2. *Mol Pharmacol.* 2006;69:66-75.
21. Conde E, Angulo B, Tang M, Morente M, Torres-Lanzas J, Lopez-Encuentra A, et al. Molecular context of the EGFR mutations: evidence for the activation of mTOR/S6K signaling. *Clin Cancer Res.* 2006;12:710-7.
22. Salvucci O, Bouchard A, Baccarelli A, Deschenes J, Sauter G, Simon R, et al. The role of CXCR4 receptor expression in breast cancer: a large tissue microarray study. *Breast Cancer Res Treat.* 2006;97:275-83.
23. Fujii N, Nakashima H, Tamamura H. The therapeutic potential of CXCR4 antagonists in the treatment of HIV. *Expert Opin Investig Drugs.* 2003;12:185-95.
24. Liles WC, Broxmeyer HE, Rodger E, Wood B, Hubel K, Cooper S, et al. Mobilization of hematopoietic progenitor cells in healthy volunteers by AMD3100, a CXCR4 antagonist. *Blood.* 2003;102:2728-30.