RBM5 as a Putative Tumor Suppressor Gene for Lung Cancer

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Abstract: *RBM5* is one member of a group of structurally related genes that includes *RBM6* and *RBM10*. *RBM10* maps to Xp11.23, and one allele is inactivated as a result of X chromosome inactivation. Both *RBM5* and *RBM6* map to 3p21.3, a tumor suppressor region that experiences loss of heterozygosity in the majority of lung cancers. Overexpression of *RBM5*, which encodes an RNA-binding protein involved in the regulation of alternative splicing and retards ascites associated tumor growth in immunocompromised mice, a phenomenon that may be related to an associated ability to modulate apoptosis. As part of our quest to gain a better understanding of how the proapoptotic activity of RBM5 might contribute to tumor suppressor function, we reviewed all the literature relating to RBM5 expression, with a focus on lung cancer. On the basis of the existing data, we suggest that—to more thoroughly assess the potential involvement of RBM5 as a lung cancer regulatory protein—more research is required regarding (a) the expression of not only full-length RBM5 but all of the alternate variants associated with the locus, in relation to histologic subtype and smoking history, and (b) the mutation status of various genes within the transforming growth factor-α signaling pathway, which may function to either directly or indirectly regulate RBM5 activity in *RBM5*-retaining lung cancers.

Key Words: RBM5, LUCA-15, Apoptosis, Tumor suppressor, Gene expression, Lung cancer.

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Tumor Suppression Relating to 3p21.3

The earliest premalignant chromosomal aberration in human lung cancers is allele loss within the short arm of chromosome 3 at 3p21.3.[1] This loss of heterozygosity occurs in practically all (>95%) small cell lung cancer (SCLC) tumors, the majority (>70%) of non-SCLC (NSCLC) tumors, and in the normal bronchial epithelium of some smokers and former smokers.[2–4] The smallest lung cancer-specific deletion region (based on homozygous deletion studies relating to 3 established SCLC cell lines (GLC20, NCI-H740, and NCI-H1450) spans 370 kb, 19 genes (RBM6, RBM5, SEMA3F, GNAT1, NAT1/SLC38A3/G17, GNAI2, SEMA3B, IFRD2, HYAL3, FUS2/NAT6, HYAL1, HYAL2, FUS1/TUSC2, RASSF1/123F2, BLU/ZMYND10, NPRL2/TUSC4, 101F6/TSP10/CYB561D2, PL6/TMEM115, and CACNA2D2), and 3 open reading frames encoding hypothetical proteins (LOC100129060, LOC100287609, and C3orf45/FLJ38608).[5] Most of the 19 genes demonstrate varying degrees of tumor suppressor activity (related to the control of processes such as cell differentiation, proliferation, signal transduction, and apoptosis), and it has been suggested that all function together as a large, integrated, biologically functionally diverse tumor suppressor unit.[3]

RBM5 (previously referred to as g15, LUCA-15, and H37) is an RNA-binding protein that has the ability to modulate apoptosis.[6–13] As shown in Figure 1*A*, RBM5-mediated apoptosis is associated with up-regulation of the proapoptotic protein BAX, down-regulation of the antiapoptotic proteins BCL-2 and BCL-X_L, increased release of mitochondrial cytochrome *c* into the cytosol and increased activation of caspases 9 and 3.[9,11–13] RBM5 modulates apoptosis by regulating the alternative splicing of apoptosis-associated premRNAs, such as CASP2 and FAS/CD95.[14,15] Although previously dismissed as unlikely to be a tumor suppressor gene, based on its lack of mutations and continued expression in most lung cancers, it has since been established that *RBM5* is not alone in this characteristic, because mutations seem to be rare in the 19 genes mapping to the common deletion region.[1,3,16] The fact that RBM5 was recently shown to retard tumor growth when overexpressed, albeit from a strong viral promoter, in either A9 mouse fibrosarcoma cells or A549 lung adenocarcinoma cells injected intraperitoneally into immunocompromised mice, suggests that it possesses at least some tumor suppressor activity.[13,17,18] In addition, multiple protein isoforms of RBM5 exist, each possessing apoptosis modulatory activity, a function not inconsistent with tumor suppressor activity.[19] (Notably, many of the other 19 genes mapping to the common deletion region, including *FUS1*,[20] *RASSF1A*,[21] *SEMA3B*,[22] *SEMA3F*,[16] *HYAL1*,[16] and *CACNA2D2*,[23] have the ability to modulate apoptosis.) Finally, *RBM5* is 1 of 9 down-regulated genes in the 17-gene

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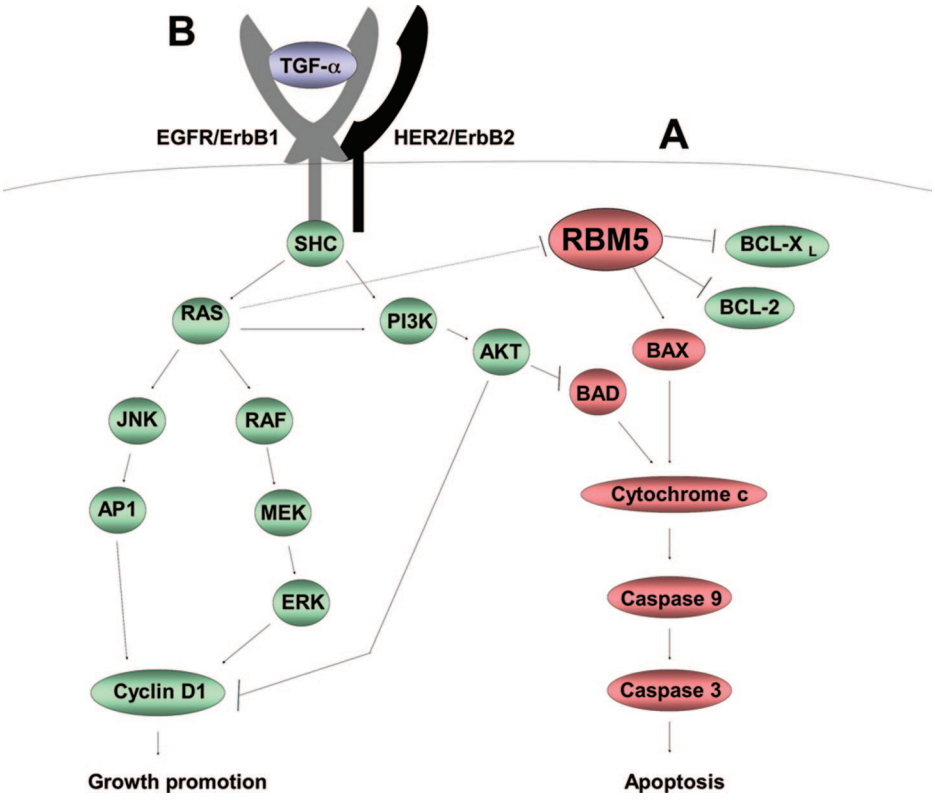

FIGURE 1. The relationship between transforming growth factor (TGF)-α and RBM5 signaling and the modulation of apoptosis. *A*, Proapoptotic activity associated with RBM5 overexpression is associated with down-regulation BCL-X_L and up-regulation of BAX expression, and antisense RBM5 overexpression is associated with the up-regulation of BCL-2 expression. Overexpressed RBM5 increases cytochrome *c* release from the mitochondria and activation of caspases 9 and 3. *B*, Activating mutations in epidermal growth factor receptor (EGFR), HER2, or KRAS result in increased growth and decreased apoptosis. For instance, RBM5 expression is down-regulated by the RAS activating mutant RAS (G12V), which would result in apoptosis inhibition. Likewise, increased EGFR and HER2 activit ies can lead to phosphorylation—and subsequent inhibition of—the proapoptotic activity associated with the BCL2-associated agonist of cell death, BAD.

metastatic signature for solid tumors (including lung) in humans and mice, suggesting that down-regulation of the protein encoded by *RBM5* is important for tumor establishment and/or progression and in a wide range of cancers.[24,25]

Two smaller deletion regions at 3p21.3 have been described after analyses of homozygous deletions common to different cancer types. One deletion region, defined by Senchenko et al.,[26] was common to lung cancer cell lines, renal cell carcinoma, and breast cancer biopsy samples; it included the 17 genes listed earlier but excluded *RBM5* and *RBM6*. The second deletion region, defined by Minna et al.,[27] was common to SCLC and breast cancer cell lines; it included only 9 of the 19 genes defined by the 3 SCLC homozygous deletions mentioned earlier and excluded *RBM6*, *FUS2*, and all the genes between, including *RBM5*. Although these data suggest that a subset of the deleted genes is associated with SCLCs and might suffice for disease progression, the contribution to malignant transformation of each of the 19 genes within the larger deletion region that is common to the 3 lung cancer cell lines, either individually or in tandem with other genes, cannot be ruled out.

RBM5 Expression

Because loss of heterozygosity at 3p21.3 is more frequent than loss of homozygosity, it means that expression of genes mapping to this tumor suppressor region is theoretically detectable, unless expression of the remaining allele is down-regulated by a process such as promoter hypermethylation. As expected, RBM5 expression is detectable in most lung cancer cell lines and primary tissues (Table 1). The question to be answered is how is the proapoptotic and potential tumor suppressive activity of the remaining *RBM5* allele silenced in lung cancers?

Inactivation of potential tumor suppressor activity can be attributed to haploinsufficiency, but is more commonly a result of gene mutation or transcriptional inactivation through promoter hypermethylation (such as is the case for the 3p21.3 genes *RASSF1A*, *BLU*, *SEMA3F,* and *SEMA3B* in lung cancers).[3,28] As is the case for the *FUS1* tumor suppressor gene that maps to 3p21.3, only a few *RBM5* mutations have been observed in lung cancer tumors, and *RBM5* promoter hypermethylation does not seem to account for any observed reduced or absent expression.[1,28–30] This suggests that, like *FUS1*, *RBM5* expression and function in lung cancers are regulated at another level.

Before we conclude that promoter hypermethylation is not responsible for reduced RBM5 expression levels in lung cancer, however, it should be noted that there is evidence that promoter hypermethylation is related to both histologic subtype and smoking exposure.[31] Promoter hypermethylation of specific genes was noted with a higher frequency in lung adenocarcinomas than squamous cell lung cancers.[31] Within the adenocarcinoma subtype, promoter hypermethylation was more frequent in ever smokers than never smokers, and within the ever smoker subgroup, promoter hypermethylation was more frequent in current smokers than in former smokers.[31] In the *RBM5* promoter hypermethylation study, neither histologic subtype nor smoking history was considered.[28] With reference to an earlier study by Oh et al.,[17] however, it

TABLE 1. RBM5 Gene Expression in Lung Cancer Cell Lines and Primary Tumor Tissue Samples

Histological Subtype	Characteristics	Cell Lines (mRNA)				Primary Tissue			
		RBM5 (+)	Reference	RBM5 (−)	Reference	mRNA	Reference	Protein	Reference
SCLC	3p21.3 (−) smokers	NCI-H82	1	GLC20	1				
		NCI-H146	1	H740	1, 17				
		NCI-H249	1	H1450	1				
		NCI-H524	1						
		NCI-H1514	1						
		NCI-H1618	1						
		NCI-H2141	1						
		NCI-H2171	1						
		NCI-H2227	1						
		GLC1	29						
		GLC2	29						
		GLC3	29						
		GLC4	29						
		GLC7	29						
		GLC8	29						
		GLC16	29						
		GLC34	29						
		GLC35	29						
		GLC36	29						
		GLC42	29						
		GLC45	18, 29						
Adenocarcinoma	3p21.3 (−)/3p21.3 (+) smokers and never smokers	NCI-H358	1, 13			4 of 5 ↓	17	32 of 39 ↓	17
		NCI-H838	1						
		NCI-H1435	Sutherland, unpublished						
		NCI-H1742	1						
		NCI-H1793	Sutherland, unpublished						
		NCI-H2077	1						
		A549	13						
		GLCA2	29						
Squamous	3p21.3 (−) smokers	SK-MES-1	13			5 of 5 ↓	17	11 of 17 ↓	17
		NCI-H520	13						
Large cell		NCI-H460	1, 13			1 of 1 ↑	17	3 of 6 ↓	17
		NCI-H1155	1						
		NCI-H1299	1, 13						
Mesothelioma		NCI-H290	1						
		NCI-H2052	1						

↓, down-regulated; ↑, up-regulated; SCLC, small cell lung cancer.

would appear that the samples used in the promoter hypermethylation study consisted of six squamous tumor samples, four adenocarcinomas, and one large cell carcinoma. The six tumor samples with the most significantly reduced RBM5 mRNA levels were of the squamous type (more often than not, associated with 3p21.3 loss and smoking, and not as frequently associated with promoter hypermethylation),[1,31,32] whereas three of the nine with the less significantly reduced RBM5 mRNA levels were adenocarcinomas (associated with both smokers and never smokers, but only 50% of the time associated with 3p21.3 loss,[1] and more frequently associated with promoter hypermethylation[31]). The one tumor sample with no change in RBM5 mRNA expression compared with its nontumor counterpart was an adenocarcinoma, whereas the one tumor sample that had more *RBM5* mRNA than its nontumor counterpart was a large cell carcinoma. These results would certainly suggest that *RBM5* gene expression is related to histologic subtype in NSCLC and may be related to smoking history. The results also suggest that promoter hypermethylation cannot be ruled out as an *RBM5* inhibitory mechanism, because the absence of promoter hypermethylation recorded by Oh et al. may have been related to 3p21.3 homozygous deletions in at least three of the squamous tumor samples (generally smoking associated) and a nonsmoking-related tumorigenic mechanism in the

adenocarcinomas. We would argue that smoking history should be investigated before definitively ruling out promoter hypermethylation as a mechanism of RBM5 expression level reduction in lung cancer.

Other than these 11 matched lung tissue samples, the only reports concerning RBM5 mRNA expression levels in lung cancer involve cell lines, most of which seem to express RBM5 at levels equivalent to levels observed in nontumor cells, a phenomenon that might be related to long-term culture-associated alterations in gene expression.[1,13,18,26,29] A single study, also by Oh et al.,[17] examined the levels of RBM5 protein: in a range of NSCLC primary tumor samples, the levels varied but were generally decreased compared with nontumor samples. Sixty-five percent and 82% of the squamous cell carcinoma and adenocarcinoma samples, respectively, showed a strong reduction in protein expression.

Notably, all mutation and expression studies relating to *RBM5*, including Northern blotting, RT-PCR, and immunohistochemistry (refer to Table 1 for references), focused on one isoform. RBM5 premRNA is alternatively spliced to produce at least three protein coding variants, some with opposing apoptotic functions.[6,7,10] In addition, at least one antisense noncoding mRNA is transcribed within the *RBM5* gene locus.[33] Finally, differential expression of transcription-induced chimeras of *RBM6* and *RBM5* has been observed in tumor tissue compared with nontumor tissue and among tumor types.[34] Regulation of *RBM5* gene expression is, therefore, a complex process that should also be examined in relation to histologic subtype and smoking history to more clearly define the purported tumor suppressor role of RBM5.

Smoking Status, TGF-α Signaling, and RBM5

Recent studies suggest that although all NSCLCs seem to involve alterations in the transforming growth factor (TGF)-α pathway (Figure 1*B*), the alterations are likely to be different depending on smoking exposure history.[35] For instance, epidermal growth factor receptor (*EGFR*) activating mutations are more often associated with never smoker-related lung cancers, whereas *KRAS* activating mutations are more often associated with smoking-related NSCLC. Interestingly, RBM5 is downregulated by the constitutively activated RAS mutant protein, RAS(G12V), in rat embryonic fibroblast cells.[36] In light of these observations, it would perhaps be prudent to examine the relationship between activating *EGFR* mutations or activating *KRAS* mutations and *RBM5* gene expression or function in lung cancer. In addition, one of a number of potential EGFR binding partners, the proto-oncoprotein HER2/ErbB2, seems to be overactive in a small percentage of both SCLC and never smoker-related NSCLC.[37,38] These activating mutations in *EGFR*, *KRAS*, and *HER2* are mutually exclusive events.[39,40] Interestingly, HER2 overexpression has been shown to affect the alternative splicing of RBM5.[41] In light of these recent advances regarding lung cancer signaling, we would like to suggest that future studies relating to RBM5 expression and potential tumor suppressor activity take into consideration not only the histologic subtype but also the smoking history and mutation status of genes within the TGF-α signaling pathway to get a more accurate assessment of the role played by RBM5 in lung cancer initiation and/or progression. In light of recent trends toward tailoring therapies to tumor subtype and/or genotype, this new direction for putative tumor suppressor gene functional analysis seems warranted.

REFERENCES

1. Lerman MI, Minna JD. The 630-kb lung cancer homozygous deletion region on human chromosome 3p21.3: identification and evaluation of the resident candidate tumor suppressor genes. The International Lung Cancer Chromosome 3p21.3 Tumor Suppressor Gene Consortium. *Cancer Res* 2000;60:6116–6133.
2. Kok K, Naylor SL, Buys CH. Deletions of the short arm of chromosome 3 in solid tumors and the search for suppressor genes. *Adv Cancer Res* 1997;71:27–92.
3. Ji L, Minna JD, Roth JA. 3p21.3 tumor suppressor cluster: prospects for translational applications. *Future Oncol* 2005;1:79–92.
4. Wistuba II, Behrens C, Virmani AK, et al. High resolution chromosome 3p allelotyping of human lung cancer and preneoplastic/preinvasive bronchial epithelium reveals multiple, discontinuous sites of 3p allele loss and three regions of frequent breakpoints. *Cancer Res* 2000;60:1949–1960.
5. Wei MH, Latif F, Bader S, et al. Construction of a 600-kilobase cosmid clone contig and generation of a transcriptional map surrounding the lung cancer tumor suppressor gene (TSG) locus on human chromosome 3p21.3: progress toward the isolation of a lung cancer TSG. *Cancer Res* 1996;56:1487–1492.
6. Sutherland LC, Edwards SE, Cable HC, et al. LUCA-15-encoded sequence variants regulate CD95-mediated apoptosis. *Oncogene* 2000;19:3774–3781.
7. Rintala-Maki ND, Sutherland LC. LUCA-15/RBM5, a putative tumour suppressor, enhances multiple receptor-initiated death signals. *Apoptosis* 2004;9:475–484.
8. Rintala-Maki ND, Abrasonis V, Burd M, et al. Genetic instability of RBM5/LUCA-15/H37 in MCF-7 breast carcinoma sublines may affect susceptibility to apoptosis. *Cell Biochem Funct* 2004;22:307–313.
9. Mourtada-Maarabouni M, Sutherland LC, Williams GT. Candidate tumour suppressor LUCA-15 can regulate multiple apoptotic pathways. *Apoptosis* 2002;7:421–432.
10. Mourtada-Maarabouni M, Sutherland LC, Meredith JM, et al. Simultaneous acceleration of the cell cycle and suppression of apoptosis by splice variant delta-6 of the candidate tumour suppressor LUCA-15/RBM5. *Genes Cells* 2003;8:109–119.
11. Sutherland LC, Lerman M, Williams GT, et al. LUCA-15 suppresses CD95-mediated apoptosis in Jurkat T cells. *Oncogene* 2001;20:2713–2719.
12. Sutherland LC, Lerman M, Williams GT, et al. LUCA-15 suppresses CD95-mediated apoptosis in Jurkat T cells. *Oncogene* 2004;23:629.
13. Oh JJ, Razfar A, Delgado I, et al. 3p21.3 tumor suppressor gene H37/Luca15/RBM5 inhibits growth of human lung cancer cells through cell cycle arrest and apoptosis. *Cancer Res* 2006;66:3419–3427.
14. Fushimi K, Ray P, Kar A, et al. Up-regulation of the proapoptotic caspase 2 splicing isoform by a candidate tumor suppressor, RBM5. *Proc Natl Acad Sci USA* 2008;105:15708–15713.
15. Bonnal S, Martinez C, Forch P, et al. RBM5/Luca-15/H37 regulates Fas alternative splice site pairing after exon definition. *Mol Cell* 2008;32:81–95.
16. Zabarovsky ER, Lerman MI, Minna JD. Tumor suppressor genes on chromosome 3p involved in the pathogenesis of lung and other cancers. *Oncogene* 2002;21:6915–6935.
17. Oh JJ, West AR, Fishbein MC, et al. A candidate tumor suppressor gene, H37, from the human lung cancer tumor suppressor locus 3p21.3. *Cancer Res* 2002;62:3207–3213.
18. Ter Elst A, Hiemstra BE, van der Vlies P, et al. Functional analysis of lung tumor suppressor activity at 3p21.3. *Genes Chromosomes Cancer* 2006;45:1077–1093.
19. Viktorsson K, Lewensohn R. Apoptotic signaling pathways in lung cancer. *J Thorac Oncol* 2007;2:175–179.

20. Ji L, Roth JA. Tumor suppressor FUS1 signaling pathway. *J Thorac Oncol* 2008;3:327–330.
21. Agathanggelou A, Bieche I, A hmed-Choudhury J, et al. Identification of novel gene expression targets for the Ras association domain family 1 (RASSF1A) tumor suppressor gene in non-small cell lung cancer and neuroblastoma. *Cancer Res* 2003;63:5344–5351.
22. Castro-Rivera E, Ran S, Thorpe P, et al. Semaphorin 3B (SEMA3B) induces apoptosis in lung and breast cancer, whereas VEGF165 antagonizes this effect. *Proc Natl Acad Sci USA* 2004;101:11432–11437.
23. Carboni GL, Gao B, Nishizaki M, et al. CACNA2D2-mediated apoptosis in NSCLC cells is associated with alterations of the intracellular calcium signaling and disruption of mitochondria membrane integrity. *Oncogene* 2003;22:615–626.
24. Ramaswamy S, Ross KN, Lander ES, et al. A molecular signature of metastasis in primary solid tumors. *Nat Genet* 2003;33:49–54.
25. Qiu TH, Chandramouli GV, Hunter KW, et al. Global expression profiling identifies signatures of tumor virulence in MMTV-PyMT-transgenic mice: correlation to human disease. *Cancer Res* 2004;64: 5973–5981.
26. Senchenko VN, Liu J, Loginov W, et al. Discovery of frequent homozygous deletions in chromosome 3p21.3 LUCA and AP20 regions in renal, lung and breast carcinomas. *Oncogene* 2004;23:5719–5728.
27. Sekido Y, Ahmadian M, Wistuba II, et al. Cloning of a breast cancer homozygous deletion junction narrows the region of search for a 3p21.3 tumor suppressor gene. *Oncogene* 1998;16:3151–3157.
28. Oh JJ, Boctor BN, Jimenez CA, et al. Promoter methylation study of the H37/RBM5 tumor suppressor gene from the 3p21.3 human lung cancer tumor suppressor locus. *Hum Genet* 2008;123:55–64.
29. Timmer T, Terpstra P, van den Berg A, et al. A comparison of genomic structures and expression patterns of two closely related flanking genes in a critical lung cancer region at 3p21.3. *Eur J Hum Genet* 1999;7: 478–486.
30. Oh JJ, Koegel AK, Phan DT, et al. The two single nucleotide polymorphisms in the H37/RBM5 tumour suppressor gene at 3p21.3 correlated with different subtypes of non-small cell lung cancers. *Lung Cancer* 2007;58:7–14.
31. Toyooka S, Maruyama R, Toyooka KO, et al. Smoke exposure, histologic type and geography-related differences in the methylation profiles of non-small cell lung cancer. *Int J Cancer* 2003;103:153–160.
32. Todd S, Franklin WA, Varella-Garcia M, et al. Homozygous deletions of human chromosome 3p in lung tumors. *Cancer Res* 1997; 57:1344–1352.
33. Rintala-Maki ND, Sutherland LC. Identification and characterisation of a novel antisense non-coding RNA from the RBM5 gene locus. *Gene* 2009;445:7–16.
34. Wang K, Ubriaco G, Sutherland LC. RBM6-RBM5 transcription-induced chimeras are differentially expressed in tumours. *BMC Genomics* 2007;8:348.
35. Gazdar AF, Shigematsu H, Herz J, et al. Mutations and addiction to EGFR: the Achilles 'heal' of lung cancers? *Trends Mol Med* 2004;10: 481–486.
36. Edamatsu H, Kaziro Y, Itoh H. LUCA15, a putative tumour suppressor gene encoding an RNA-binding nuclear protein, is down-regulated in ras-transformed Rat-1 cells. *Genes Cells* 2000;5:849–858.
37. Canoz O, Ozkan M, Arsav V, et al. The role of c-erbB-2 expression on the survival of patients with small-cell lung cancer. *Lung* 2006; 184:267–272.
38. Hirsch FR, Franklin WA, Veve R, et al. HER2/neu expression in malignant lung tumors. *Semin Oncol* 2002;29:51–58.
39. Buttitta F, Barassi F, Fresu G, et al. Mutational analysis of the HER2 gene in lung tumors from Caucasian patients: mutations are mainly present in adenocarcinomas with bronchioloalveolar features. *Int J Cancer* 2006;119:2586–2591.
40. Bae NC, Chae MH, Lee MH, et al. EGFR, ERBB2, and KRAS mutations in Korean non-small cell lung cancer patients. *Cancer Genet Cytogenet* 2007;173:107–113.
41. Rintala-Maki ND, Goard CA, Langdon CE, et al. Expression of RBM5-related factors in primary breast tissue. *J Cell Biochem* 2007;100:1440–1458.