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Growth Factors Regulate Heterogeneous Nuclear Ribonucleoprotein K Expression and Function*

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Epidermal growth factor (EGF) family of growth factors and their receptors regulate normal and cancerous epithelial cell proliferation, a process that can be suppressed by antireceptor blocking antibodies. To identify genes whose expression may be modulated by antireceptor blocking antibodies, we performed a differential display screen with cells grown in the presence or absence of antireceptor blocking antibodies; isolates from one cDNA clone were 100% identical to human heterogeneous nuclear ribonucleoprotein K (hnRNP K), a protein with a conserved KH motif and RGG boxes, has been implicated in such functions as sequence-specific DNA binding, transcription, RNA binding, and nucleocytoplasmic shuttling. Both EGF and heregulin-β1 induced expression of hnRNP K mRNA and protein in human breast cancer cells. This growth factor-mediated hnRNP K expression was effectively blocked by pretreatment of cultures with humanized anti-EGF receptor (EGFR) antibody C225, or anti-human epidermal growth factor receptor-2 (HER2) antibody. Anti-EGFR monoclonal antibody also caused regression of human tumor xenografts and reduction in hnRNP K levels in athymic mice. Samples from grade III human breast cancer contained more hnRNP K protein than samples from grade II cancer. Finally, overexpression of hnRNP K in breast cancer cells significantly increased target c-*myc* promoter activity and c-Myc protein, hnRNP K protein levels, and enhanced breast cancer cell proliferation and growth in an anchorage-independent manner. These results suggested that the activity of human EGF receptor family members regulates hnRNP K expression by extracellular growth promoting signals and that therapeutic humanized antibodies against EGFR and HER2 can effectively block this function.

Growth factors and their receptors play an important role in regulating proliferation of epithelial cells. Abnormalities in the expression, structure, or activity of their proto-oncogene products contribute to the development and pathogenesis of cancer. For example, the human epidermal growth factor receptor, HER1,[1] is overexpressed in a large number of epithelial tumor cells (1). HER2, the second member of the HER family, shares extensive sequence homology with the tyrosine kinase domain of HER1 (1–3) and is overexpressed, amplified, or both in a number of human malignancies, including breast. Recently, HER3 and HER4 have been added to this receptor family (2, 3). Regulation of these receptor family members is complex, and they can be trans-activated in a ligand-dependent manner. For example, binding of heregulin-β1 (HRG) to HER3 or HER4 can activate the HER2 as a result of HER2/HER3 or HER4/HER2 heterodimeric interactions (2–4). HER1 and HER2 have been shown to induce transformation in recipient cells (2–4), possibly because of excessive activation of signal transduction pathways. In contrast, transformation by HER3 or HER4 requires the presence of HER1 or HER2 (5).

Since growth factors regulate the proliferation of cancer cells by activating cell-surface receptors, one approach to controlling cell proliferation is to use antireceptor-blocking monoclonal antibodies to interfere with growth factor receptor-mediated autocrine or paracrine growth stimulation (6). C225, the human murine chimeric antibody against the EGF receptor (EGFR), blocks binding of ligand and prevents ligand-induced activation of receptor tyrosine kinase (6, 7). C225 therapy has been success in phase I and phase IIA multicenter clinical trials in combination with chemotherapy or radiation (8–11). Similarly, the humanized form of anti-HER2 recombinant monoclonal antibody (HCT) inhibits the growth of breast cancer cells overexpressing HER2 (12) and is currently being used as an effective drug against breast cancer both alone (13) and in combination with chemotherapy (14). Antireceptor antibodies inhibit many processes, including mitogenesis, cell-cycle progression, invasion and metastasis, angiogenesis, and DNA repair (8).

In eukaryotic cells, heterogeneous nuclear RNAs (hnRNAs), from which mRNAs are generated by RNA processing, associate with specific nuclear proteins to form large hnRNP complexes (15, 16). These hnRNP proteins bind pre-mRNAs and are believed to play important roles in mRNA biogenesis (17, 18), nucleocytoplasmic transport of mRNA (19–21), and cytoplasmic mRNA trafficking (22). To date, about 20 hnRNPs (A through U) have been identified. HnRNP K, the major poly(iC)-binding protein, has several structural features not shared

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[1] The abbreviations used are: HER, *h*uman *e*pidermal growth factor *r*eceptor; HRG, heregulin-β1; EGF, epidermal growth factor; EGFR, EGF receptor; C225, anti-EGF receptor antibody; mAb, monoclonal antibody; HCT, anti-HER2 recombinant-derived humanized mAb. hnRNP K, heterogeneous nuclear ribonucleoprotein K; GDA, gene discovery array; KH, K homology; PAGE, polyacrylamide gel electrophoresis; TGF, transforming growth factor.

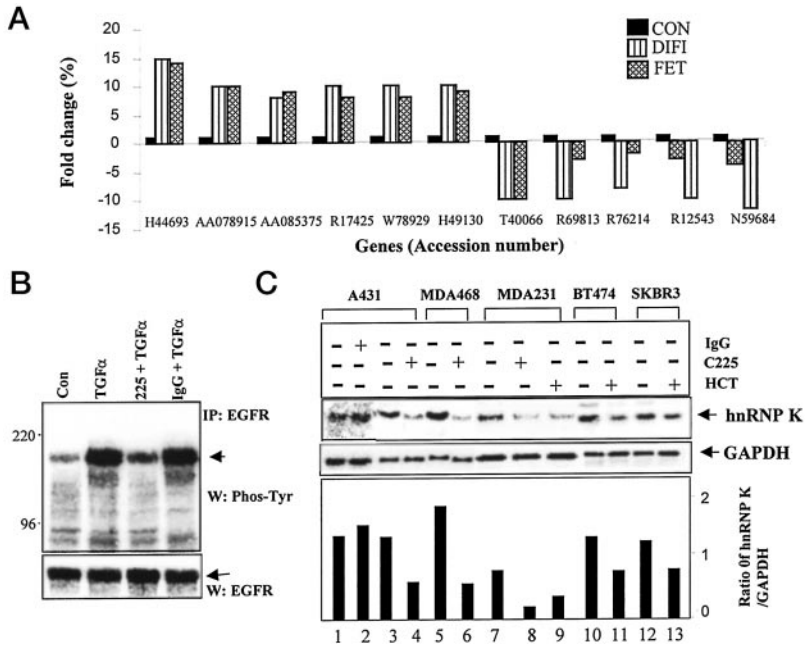

FIG. 1. **Identification of hnRNP K as an antireceptor antibody-regulated gene.** *A*, representative differential display patterns of antireceptor-regulated spots on the GDA filters. Human colon DiFi and FET cells were treated with C225 (50 nM) for 10 h. Total RNAs were isolated and used for hybridization with the GDA array filters, as described under "Experimental Procedures." Results are presented as percent change by antibody treatment over control (*CON*) untreated cells. *B*, MDA231 cells were treated with or without 30 nM TGF-α in the absence or presence of 30 nM C225 or IgG for 30 min. Cell lysates were immunoprecipitated with an anti-EGFR mAb 528 and immunoblotted with anti-phosphotyrosine mAb 4G10, and the position of EGFR is shown by an *arrow*. Sequentially, the above blot was reprobed with an anti-EGFR mAb to demonstrate the levels of EGFR in different tubes. *C*, tumor cell lines were treated with or without C225 or HCT or IgG (50 nM) for 16 h. Total RNA was isolated, and the expression of hnRNP K mRNA was detected by Northern hybridization. Results are representative of three experiments. *GAPDH*, glyceraldehyde-3-phosphate dehydrogenase.

with other hnRNP proteins (23). For example, the binding of hnRNP K to nucleic acid is mediated by three repeat motifs termed the KH (K homology) domains rather than by the consensus RNA-binding sequence found in other hnRNP proteins (23, 24). The KH domain is an evolutionarily conserved RNA-binding motif also found in fragile X protein FMR1 (25), meiosis-specific splicing factor MER1 (26), and paraneoplastic Ri autoantigen (27). In addition, hnRNP K binds single-stranded DNA *in vitro* (28) and has been identified as a sequence-specific DNA-binding protein (29), consistent with its proposed role in transcription. More recently, hnRNP K has been shown to bind to a cis-element in the human *c-myc* promoter (30) and to activate *c-myc* expression (31) by promoting the synthesis of *c-myc* mRNA from a reporter gene (32). The hnRNP K protein can also interact with some proto-oncogene products and to act as a docking platform in signal transduction cascades (33, 34). The potential involvement of hnRNP in transformation was suspected, four splice variants of hnRNP K are up-regulated in SV40-transformed cells (35), and hnRNP B1 is the only other member of the hnRNP family, beside hnRNP K that is overexpressed in human lung cancers (36). Despite the widely believed involvement of hnRNP K in post-transcriptional control, its possible regulation by the EGF family and by therapeutic antireceptor antibodies remains unexplored.

To identify genes whose expression may be modulated by the activity of the EGF family of receptors because of ligand-induced activation of receptor tyrosine kinases or inhibition of receptor-associated tyrosine kinase activation, we used a human cDNA array approach to isolate cDNAs differentially expressed in the presence and absence of antireceptor antibodies. We identified one clone that was identical to human hnRNP K. In human breast cancer cells, EGF and HRG induced hnRNP K mRNA and protein expression that could be effectively blocked by pretreatment with antireceptor monoclonal antibodies. Our results also suggested that hnRNP K is involved in EGF- or HRG-induced transcription from the c-*myc* promoter and that hnRNP K expression can be used as a molecular monitor to assess the anti-tumor action of therapeutic antireceptor agents.

EXPERIMENTAL PROCEDURES

Cell Cultures and Reagents—Human colon cancer DiFi and FET cells, and breast cancer MCF-7, MDA-468, BT-474, SK-BR3, MDA-231, and MDA-435 cells and vulvar carcinoma A431 cells, were maintained in Dulbecco's modified Eagle's medium-F-12 (1:1) supplemented with 10% fetal calf serum. C225 and HCT ware provided by Imclone Systems, Inc. and Genentech Inc., respectively. Recombinant HRG-β1 was purchased from Neomarkers, Inc. Anti-vinculin antibody and recombinant EGF was purchased from Sigma. Polyclonal and monoclonal antibodies against hnRNP K were generously provided by Drs. Pradip Raychaudhuri and Gideon Dreyfuss (32, 23).

Gene Discovery Array Screening—DiFi and FET cells were cultured with or without C225 for 10 h, and total RNA was isolated using TRIZOL reagent (Life Technologies, Inc.). Poly(A) RNA was isolated using a poly(A) RNA isolation kit (Invitrogen). Gene discovery array (GDA) filters (version 1.2) containing 18,376 nonredundant human cDNA clones were purchased from Genome Systems. Probe preparation and hybridization conditions were performed according to manufacturer's recommendations. In brief, 2 μg of poly(A) RNA was reverse-transcribed using T18MN primer (Genome Systems) and Superscript II reverse transcriptase (Life Technologies, Inc.) in the presence of [^{33}P]dCTP. cDNA was purified using a G-50 spin column, treated with 0.25 M NaOH to remove RNA, and neutralized with Tris. Identical filters were hybridized to cDNA probes in 50% formamide buffer at 42 °C for 16 h, washed two times with 2× SSC, and washed two more with 0.6× SSC. Filters were exposed to a phosphoimager, and images were quantitated by the bioinformatics department at Genome Systems using Array Vision software. Normalized intensity values for control and C225-treated filters were compared with determine -fold induction.

Cell Extracts, Immunoblotting, and Immunoprecipitation—To prepare cell extracts, cells were washed three times with phosphate-buffered saline and lysed in buffer (50 mM Tris-HCl, pH 7.5, 120 mM NaCl, 0.5% Nonidet P-40, 100 mM NaF, 200 mM $NaVO_5$, 1 mM PMSF, 10 μg/ml leupeptin, 10 μg/ml aprotinin) for 15 min on ice. The lysates were centrifuged in an Eppendorf centrifuge at 4 °C for 15 min. Cell lysates

containing equal amounts of protein were resolved by SDS-PAGE, transferred to nitrocellulose, and probed with the appropriate antibodies. Equal numbers of cells were metabolically labeled for 4–8 h with 100 μCi/ml [^{35}S]methionine in methionine-free medium containing 2% dialyzed fetal bovine serum in the absence or presence of indicated treatments. Cell extracts containing equal trichloroacetic acid perceptible counts were resolved on SDS-PAGE, immunoprecipitated with the desired antibody, and analyzed after exposure to film.

Northern Hybridization—Total cytoplasmic RNA was isolated using the TRIZOL reagent, and 20 μg of RNA was analyzed by Northern hybridization using a cDNA fragment of human hnRNP K. Ribosomal RNA (28 and 18 S) was used to assess the integrity of the RNA and for RNA-loading controls; blots were also routinely reprobed with control glyceraldehyde-3-phosphate dehydrogenase cDNA.

Human Tissue Samples—Human breast tissue samples were obtained from a tissue bank maintained by Dr. Luis Costa (Hospital de Santa Maria, Lisbon, Portugal). Specimens derived from patients who had undergone surgery for breast cancer were frozen in liquid nitrogen and stored at −80 °C, as described previously (37). Thawed tissue samples were homogenized in Triton X-100 lysis buffer (20 mM HEPES, 150 mM NaCl, 1% Triton X-100, 0.1% deoxycholate (v/w), 2 mM EDTA, 2 mM EDTA, 2 mM sodium orthovanadate, and protease inhibitor mixture (Roche Molecular Biochemicals), and equal amounts of proteins were analyzed by Western blotting.

Xenografts Studies—A431 cells (10^7) were implanted subcutaneously into nude mice and allowed to grow for 8 days. The mice were given intraperitoneal injections of either phosphate-buffered saline or C225, 1 mg/mouse, twice a week for 3 weeks. Treatment of C225 alone resulted in transient inhibition of tumor growth. Tumor size was measured twice weekly with calipers, using the formula $\pi/6x$ larger diameter × (smaller diameter).

RESULTS AND DISCUSSION

Identification of hnRNP K as an Antireceptor Antibody-regulated Gene—In an attempt to identify genes whose expression may be modulated in human cancer cells by antireceptor antibodies that block receptor activity, we used GDA filters human version 1.2 obtained from Genome Systems. GDA is a single nylon filter spotted with 18,376 nonredundant human cDNA clones chosen from the Integrated Molecular Analysis of Genome and Expression collection. We have initially used two cell lines: high EGFR overexpressing DiFi cell line and a moderate EGFR expressing FET cell line (38). Cells were cultured with or without anti-EGF receptor antibody C225 for 10 h, total RNA was isolated, and cDNA probes were prepared using reverse transcription. The filters were hybridized and exposed to a phosphoimager. Phosphoimager scans were sent to Genome Systems, and their Bioinformatics Department used Array Vision software to analyze differential expression of genes. This analysis identified several differentially expressed gene products. Representative data for 11 genes whose expression was altered are shown in Fig. 1. To further characterize these antibody-responsive genes, we obtained expressed sequence tag clones arrayed on GDA filters from Genome Systems. While using the cDNAs obtained, we uncovered the problem that the clones received from Genome Systems did not sequence verify to data base numbers, and we got mixed results in our Northern blots. Upon contacting Genome Systems, we found that their GDA version 1.2 filters were not sequence-verified, were developed on the basis of information from Integrated Molecular Analysis of Genome and Expression consortium clones, and were expected to have mixed colony stocks. (GDA1.2 filters are discontinued due to these problems). To resolve this issue, we isolated multiple single colonies from each clone stock and analyzed them by Northern analysis. Northern analysis of single colony-isolated cDNAs showed that one of the cDNAs was down-regulated by antibody treatment. Sequencing of the cDNA revealed that it had extensive similarity with hnRNP K; however it possessed an extra region of 400 base pairs, suggesting that this was an isoform of hnRNP K. Since these expressed sequence tag clones were isolated from fetal liver, we screened a mammary gland cDNA library (CLONTECH) with the 1.1-kilobase pair cDNA probe to verify that this isoform of hnRNP K was expressed in the mammary gland. Ten positive clones were isolated and sequenced. Comparison of the sequences with the GenBank^{TM} revealed that the sequences were 100% identical to human hnRNP K gene.

To confirm that the hnRNP K gene was regulated by growth factors in mammary epithelial cells, regulation of the hnRNP K gene expression by antibodies was verified by experiments involving Northern analysis using the full-length cDNA (1.8 kilobase pairs) isolated from mammary gland (hnRNP K, identical GenBank^{TM} to accession number S74678). A431 cells, which overexpress EGFR, are growth-stimulated by autocrine transforming growth factor-α (TGF-α) and growth-inhibited by C225 (6, 7). To determine the role of growth factor receptor activity in the regulation of hnRNP, we asked whether C225 could down-regulate the steady-state level of hnRNP K mRNA.

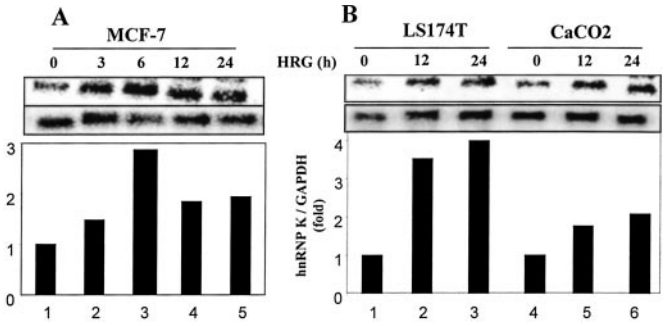

FIG. 2. **Regulation of hnRNP K mRNA expression by growth factors.** Breast cancer MCF-7 (*A*), and colon cancer LS174T and CaCO2 cells (*B*) were treated with HRG (30 nM) for the indicated times. Total RNA was isolated, and hnRNP K mRNA levels were detected by Northern blot analysis. Subsequently, the blot was reprobed with a glyceraldehyde-3-phosphate dehydrogenase (*GAPDH*) cDNA probe. Quantitation of mRNA is shown in the *bottom panels*. Results are representative of two experiments.

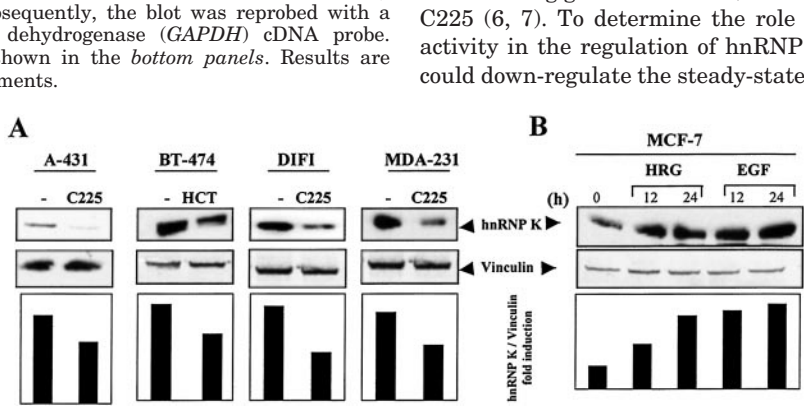

FIG. 3. **Antireceptor antibodies and growth factors modulate hnRNP K protein level.** *A*, cells were treated with C225 or HCT (50 nM) for 16 h (*A*) and EGF or HRG (30 nM) for the indicated times (*B*). Total lysates were run on SDS-PAGE and blotted with anti-hnRNP K mAb. Anti-vinculin Ab was used as an internal control. Quantitation of the ratio of hnRNP K: vinculin is shown in the *bottom panel*. Results are representative of three experiments.

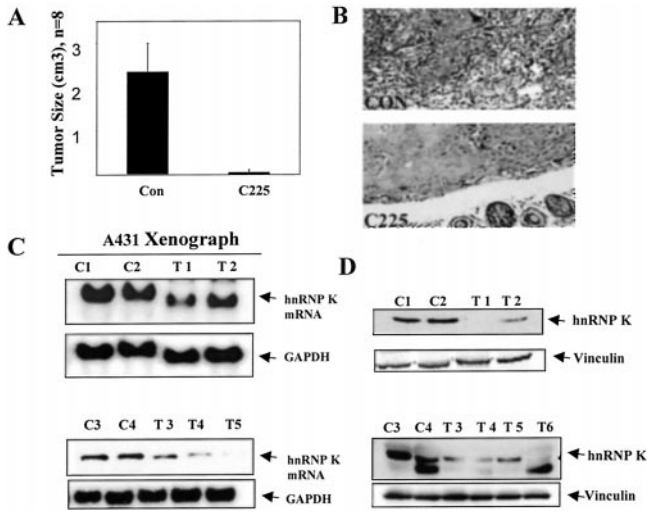

FIG. 4. **Anti-EGF receptor antibody C225 suppresses hnRNP K expression in a tumor xenograft model.** *A*, established A431 cells xenografts (0.6–0.8 cm^3) were treated with C225 (100 μg/animal, 2 times a week) or saline for 21 days. Tumor volumes in the control and C225-treated groups at day 29 are shown. *B*, histology of tumors as assessed by H&E staining. *C*, total RNA was isolated from four control and five antibody-treated tumors. The expression of hnRNP K was immediately analyzed by Northern hybridization. *GAPDH*, glyceraldehyde-3-phosphate dehydrogenase. *D*, tissue lysates from four control (*C1–C4*) and six antibody-treated (*T1–T6*) tumors were immediately immunoblotted with an anti-hnRNP mAb. The blots were reprobed with a control vinculin antibody.

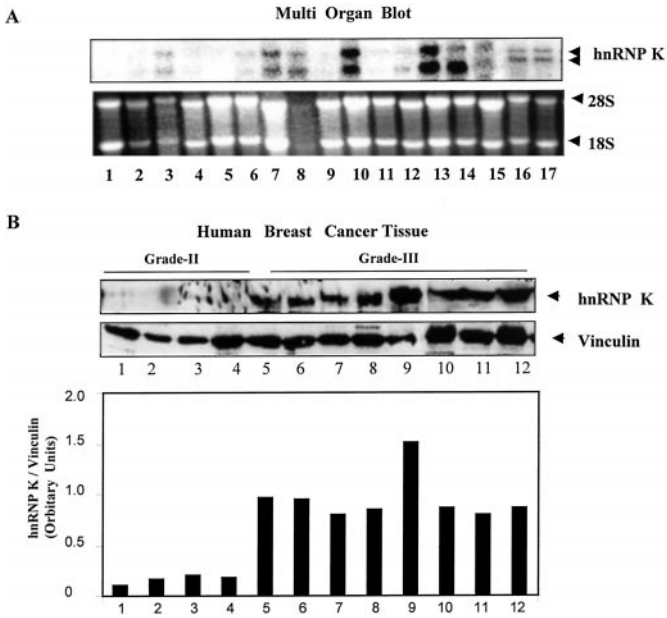

FIG. 5. **Expression of hnRNP K in multiple organs and human breast tumors.** *A*, expression profile of hnRNP K mRNA in multiple mouse organs as determined by the Northern hybridization. Ribosomal 28 S and 18 S RNAs were used to assess the integrity of RNA samples. *Lanes*: *1*, muscle; *2*, liver; *3*, heart; *4*, thymus; *5*, colon; *6*, kidney; *7*, cerebral cortex; *8*, placenta; *9*, spleen; *10*, cerebellum; *11*, uterus; *12*, stomach; *13*, ovary; *14*, testis; *15*, salivary gland; *16*, lung; *17*, adrenal gland. *B*, tissue lysates from grade II and grade III were analyzed by immunoblotting for hnRNP K expression (*upper panel*) and, subsequently, reprobed with a vinculin Ab as a loading control (*middle panel*). Quantitation of the ratio of hnRNP K: vinculin is shown in the *bottom panel*.

Earlier studies from this laboratory have shown the effectiveness of 225 antibody to inhibit TGF-α- or EGF-induced tyrosine phosphorylation of EGFR in DiFi, FET, A431, MDA468, and mouse NIH3T3 cells stably expressing human EGFR (38–41).

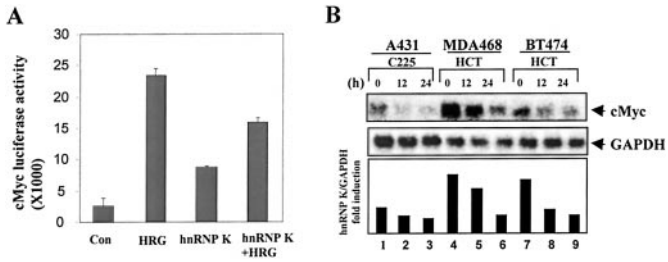

FIG. 6. **Regulation of c-Myc expression by growth factors and antireceptor antibodies.** *A*, modulation of c-Myc promoter activity by hnRNP K and HRG. MCF-7 cells were transiently transfected with a DNA for luciferase drawn by c-Myc promoter and hnRNP K or control (*Con*) DNA, and luciferase activity was measured 24 h after transfection. Some cultures were treated with HRG (30 nM) for 16 h before lysis. Relative luciferase activity is shown in the *bottom panel*. *B*, tumors cells were treated with C225 or HCT (50 nM) for 16 h, and c-Myc mRNA expression was measured by Northern hybridization. *GAPDH*, glyceraldehyde-3-phosphate dehydrogenase. Results are representative of two experiments.

Consistent with these reports, C225 also blocked the ability of EGF to stimulate the tyrosine phosphorylation of EGFR in MDA231 cells (Fig. 1*B*). Treatment of A431 or MDA468 cells with C225, but not control IgG, resulted in a decrease in hnRNP K mRNA expression (Fig. 1*C*). Similarly, HCT treatment of BT-474 or SKBR3 cells, which overexpress HER2, was associated with reduced expression of hnRNP K mRNA (Fig. 1*C*). We have earlier demonstrated the ability of anti-HER2 antibody to suppress the tyrosine phosphorylation of HER2 in BT-474 and SKBR3 cells (42).

Regulation of hnRNP K Expression by Growth Factors—Since MDA-231 cells are known to constitutively secrete HRG, a ligand for HER3 and HER4 that can transactivate EGFR and HER2, the above results raised the possibility of HRG regulation of hnRNP K mRNA expression. HRG treatment of human breast cancer MCF-7 (38), and colorectal cancer LS174 and CaCO2 cells (39), was accompanied by increased expression of hnRNP K mRNA (Fig. 2, *A* and *B*). EGF regulation of hnRNP K mRNA expression was also confirmed using a mouse NIH3T3 cell line (HER14) that stably expressed human EGFR, lack production of TGF-α, and to exogenous EGF by growth stimulation (40). EGF treatment of HER14 cells for 8 h was accompanied by a significant increase in hnRNP K mRNA levels (data not shown). Taken together, these results suggest that EGF, HRG, and antireceptor monoclonal antibodies, which interfere with EGFR and HER2, modulate hnRNP K mRNA levels in a number of cell types.

Western blot analysis was performed to determine whether the modulation of hnRNP K mRNA levels by growth factors and antireceptor monoclonal antibodies was associated with a corresponding modulation in the expression of hnRNP K protein. Results demonstrated that A431 cells and human colon carcinoma DiFi cells, which have functional TGF-α autocrine loop (38), expressed a lower level of the 65-Da hnRNP K protein, after C225 treatment (Fig. 3*A*). Similarly, HCT inhibited hnRNP K protein levels in BT-474 cells (Fig. 3*A*). In contrast, treatment of MCF-7 cells with EGF or HRG significantly increased the level of hnRNP K protein (Fig. 3*B*).

C225 Therapy Modulates hnRNP K Expression in a Xenograft Model—Our results presented above suggest that hnRNP K expression is positively regulated by growth factors and can be reduced by antireceptor-blocking antibodies. To further understand the significance of hnRNP K expression in tumor cell growth regulation, we next examined the effect of C225 therapy on the status of hnRNP K expression in *in vivo* setting using the A431 xenograft model. As expected, C225 treatment of well established A431 cell xenografts was associ-

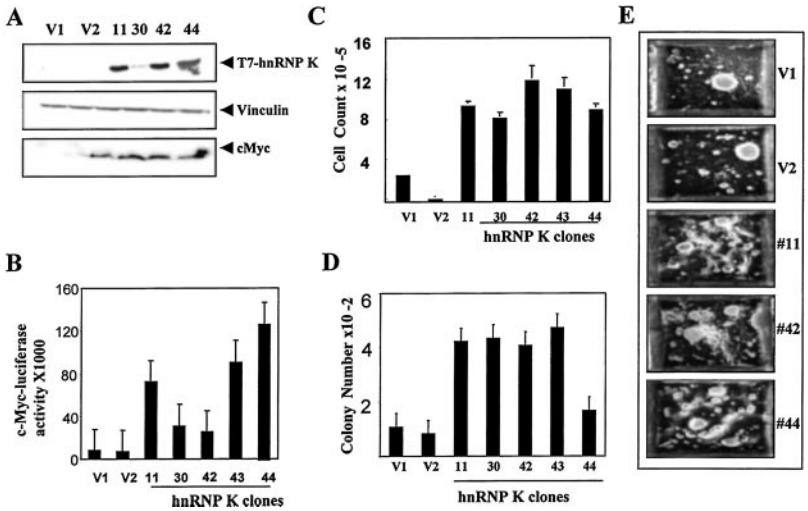

FIG. 7. **Effect of hnRNP K expression on the biology of breast cancer cells.** *A*, Western blot analysis of control and T7-tagged hnRNP K clones by anti-T7 mAb. The blot was reprobed with an anti-vinculin Ab, as a loading control, and, subsequently, with an anti-cMyc mAb. *B*, effect of T7-hnRNP K expression on the activity of the c-Myc promoter-luciferase. *C*, effect of T7-hnRNP K expression on the growth rate of cells by MTT assay. *D*, effect of T7-hnRNP K expression on anchorage-independent growth of MCF-7 cells. *E*, representative photographs of soft agar colonies ($n = 3$).

ated with a reduced regression of tumors (Fig. 4*A*) and appearance of very smooth boundaries between tumors and surrounding tissues compared with control untreated tumors (Fig. 4*B*). Importantly, significant reductions in the levels of hnRNP K mRNA (Fig. 4*C*) and hnRNP K protein (Fig. 4*D*) accompanied C225 therapy.

hnRNP K Expression in Human Breast Cancer—Since these results show that hnRNP K expression is regulated by growth factors, we explored whether there is any relationship between the proliferation state in murine and human tissue and hnRNP K expression. Northern blot analysis of multiple mice tissues revealed the presence of low levels of hnRNP K mRNA in all tissues. However, the hnRNP K transcript levels were significantly higher in tissues such as cerebellum, ovary, and testis (Fig. 5*A*). Interestingly, there was expression of only the faster migrating form of hnRNP K mRNA in testes (Fig. 5*A*, *lane 14*). We also examined the expression of hnRNP K in a small number of human breast carcinoma biopsy samples (37). In general, grade III tumor specimens had a higher level of hnRNP K protein compared with grade II tumor specimens (Fig. 5*B*). Additional studies utilizing large number of clinical samples are needed to confirm these findings.

Effect of hnRNP K on the Biology of Breast Cancer Cells—Data from several recent reports indicate that c-*myc*, a growth factor-inducible early gene, is one of the downstream targets of hnRNP K (32). We verified the potential role of hnRNP K in growth factor-mediated stimulation of transcription from the c-*myc* promoter using a luciferase reporter gene containing c-Myc promoter. As expected, cotransfection of hnRNP K upregulated the c-Myc promoter activity (Fig. 6*A*). Treatment of cells with antireceptor antibodies resulted in a significant decreased level of c-*myc* mRNA (Fig. 6*B*).

To further delineate the potential contribution of hnRNP K in breast cancer cells, we next established stable MCF-7 clones expressing T7-tagged hnRNP K or control vector (Fig. 7*A*). As expected from the data in Fig. 6, cells expressing hnRNP K also exhibited an increased level of c-Myc (Fig. 7*A*) as well as stimulation of transcription from the c-*myc* promoter (Fig. 7*B*). To examine the potential influence of hnRNP K expression on the growth characteristics of breast epithelial cancer cells, we measured the proliferation rate and ability of cells to grow in an anchorage-independent manner. Expression of hnRNP K significantly enhanced the proliferation rate of MCF7 cells (Fig. 7*C*). Overexpression of hnRNP K was accompanied by a significant reproducible enhancement of the ability of cells to form larger colonies in soft agar as compared with those formed by vector transfected control cells (Fig. 7*D*). Together, these observations suggested that breast cancer cells expressing hnRNP K have altered growth characteristics.

In summary, we provide new evidence that the hnRNP K is a target of growth factor in human cancer cells and that hnRNP K positively controls the growth rate of human breast cancer cells. This conclusion is based on the following evidence: 1) up-regulation of hnRNP K mRNA and protein by both HRG and EGF in cells with low levels of EGF and HER2 receptors; 2) down-regulation of hnRNP K mRNA and protein by HCT and C225 in cells with high levels of EGF and HER2 receptors; 3) blockage of ligand-induced stimulation of hnRNP K by an antireceptor antibody in breast cancer cells with low levels of receptors; 4) a reduction in the level of hnRNP K in tumor xenografts treated with C225; 5) deregulation of hnRNP K expression cells leads to an enhancement of the proliferation and anchorage-independent growth of breast cancer cells; and finally, 6) human breast tumor specimens from grade III tumors exhibited an increased level of hnRNP-K protein compared with the levels in grade II tumors.

The molecular mechanisms by which hnRNP K affects the growth rate of human cells are not clear at the moment. hnRNP K has been shown to activate the human c-Myc promoter (32) and increases the level of c-Myc protein. Enhanced expression of c-Myc could enable this transactivation factor to stimulate its downstream targets genes, leading to cell cycle progression. In addition, hnRNP K physically interacts with several oncogene products, including members of the Src protein tyrosine kinases (33, 34), and this could also contribute to the mitogenic response. Our demonstrated growth-promoting function of hnRNP K protein in human breast epithelial cells also supports the observation of hnRNP K up-regulation in SV40-transformed cells (35). In contrast to these findings, there are reports showing a repressing effect of hnRNP on C/EBP-β-mediated activation of the *agp* gene involved in the acute phase response (43) and induction of programmed cell death in imaginal discs of *Drosophila* (44). These observations suggest that the hnRNP K may differently influence cellular functions in a cell type-specific manner.

In summary, our findings have clearly demonstrated for the

first time a potential role of hnRNP K in the actions of HER growth factors that are widely deregulated in human cancers and that ligand-dependent hnRNP K expression can be effectively inhibited with antireceptor blocking antibodies C225 and HCT. In addition, we also provide new evidence that hnRNP K may contribute to regulating target genes that lead to enhanced growth rate of cancer cells.

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