Suppression of lysyl-tRNA synthetase, KRS, causes incomplete epithelial-mesenchymal transition and ineffective cell-extracellular matrix adhesion for migration

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Abstract. The cell-adhesion properties of cancer cells can be targeted to block cancer metastasis. Although cytosolic lysyl-tRNA synthetase (KRS) functions in protein synthesis, KRS on the plasma membrane is involved in cancer metastasis. We hypothesized that KRS is involved in cell adhesion-related signal transduction for cellular migration. To test this hypothesis, colon cancer cells with modulated KRS protein levels were analyzed for cell-cell contact and cell-substrate adhesion properties and cellular behavior. Although KRS suppression decreased expression of cell-cell adhesion molecules, cells still formed colonies without being scattered, supporting an incomplete epithelial mesenchymal transition. Noteworthy, KRS-suppressed cells still exhibited focal adhesions on laminin, with Tyr397-phopshorylated focal adhesion kinase (FAK), but they lacked laminin-adhesion-mediated extracellular signal-regulated kinase (ERK) and paxillin activation. KRS, p67LR and integrin α6β1 were found to interact, presumably to activate ERK for paxillin expression and Tyr118 phosphorylation even without involvement of FAK, so that specific inhibition of ERK or KRS in parental HCT116 cells blocked cell-cell adhesion and cell-substrate properties for focal adhesion formation and signaling activity. Together, these results indicate that KRS can promote cell-cell and cell-ECM adhesion for migration.

Introduction

Cell adhesion involves adhesions comprised of intercellular cell-cell attachments and of cell extracellular matrix protein

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(ECM) interactions (1). Proper tuning of the cell adhesion can lead to efficient migration and invasion for a successful metastasis (2). Complicated processes leading to metastasis, migration and invasion should be effectively coordinated by the functions of spatial and temporal cues between cellular and microenvironmental regions (3). Furthermore, regulation of cell adhesion properties may be targeted to control the migration and invasion for cancer metastasis. Diverse signaling or adaptor proteins play critical roles in promoting cancer cells to metastasize (4). Although the signaling molecules contributing to control of the metastatic potentials are very complicated and interconnected, more molecules still need to be identified to understand the control of cellular migration.

Lysyl-tRNA synthetase, KRS, is involved in protein translation. It catalyzes the addition of amino-lysyl to peptides that are being synthesized along the mRNA codons. Although cytosolic KRS is involved in this housekeeping role, KRS at the plasma membranes functions in immune responses (5,6) and tumor metastasis (7,8), clearly indicating non-canonical biological functions besides protein translation. Phosphorylation at the Thr52 residue of KRS by p38 mitogen-activated protein kinase (MAPK) causes dissociation from the cytosolic multi-tRNA synthetase complex (MSC) and translocation to the plasma membrane, where it associates with and stabilizes a 67-kDa laminin receptor (p67LR) for migration and metastasis (7). The p67LR associates with integrin α6β1 (9) or α6β4 (10). The integrins are important for cell-extracellular matrix (ECM) adhesion involved in cell migration and invasion (11), as well as signal transduction activation leading to tyrosine phosphorylations of focal adhesion kinase (FAK), paxillin and c-Src involved in cell-ECM adhesion (12). After subcutaneous injection of cells into mice, the interaction between KRS and p67LR can be targeted to inhibit the KRS-dependent metastasis upon a subcutaneous injection of cells to mouse, indicating that KRS can be targeted to block cancer metastasis (8). Although targeting KRS has been shown to inhibit metastasis, how KRS regulates cell-cell adhesion and cell-ECM adhesion is not clearly known.

Cell-cell adhesion involves tight junctions, adherence junctions, gap junctions and desmosomes, whereas cell-ECM

adhesion occurs at focal adhesions (FAs) and hemidesmosomes (2). Homophilic or heterophilic interactions at the junctions between membrane proteins are important in the epithelial-mesenchymal transition (EMT), and loss of cell-cell adhesion and further polarity of the epithelial cells can lead to migratory cells with mesenchymal properties (13). Cell-ECM adhesion is also important in the migration and invasion processes. Acquiring new areas for attachment to the leading edges and detachment from the rear regions must be coordinated for efficient translocation of cell bodies (14). During the coordinated regulation of cell-ECM adhesion, FA dynamics are important (15). During cell adhesion involving integrin to the ECM, cells can activate intracellular signaling molecules such as RhoA GTPases, FAK, c-Src, ERKs and paxillin (16). These activations can lead to the reorganization of F-actin networks to form new integrin-ECM interactions and subsequent tractive force generation (17).

In the present study, the KRS housekeeping protein was characterized for possible regulatory roles in cell-cell adhesion and cell-ECM adhesion related to its pro-migratory functions in HCT116 colon cancer cells. KRS regulated the expression of epithelial markers, including E-cadherin and β-catenin, involved in cell-cell adhesion. It also regulated cell-laminin adhesion-mediated signaling activities for FAK, ERKs and paxillin. Suppression of KRS resulted in loss of E-cadherin and β-catenin without a disruption of cell-cell adhesion, and of cell-laminin adhesion-mediated signaling activities, leading to impaired migratory abilities. The results of the present study showed that KRS was able to regulate cell adhesion properties involved in cellular migration, suggesting that it could be a therapeutic target for treatment of colon cancer metastasis.

Materials and methods

Cells. HCT116 colon cancer cells (American Type Culture Collection, Manassas, VA, USA) were stably transfected with shRNA against KRS, as previously described (18). HCT116 cells overexpressing KRS were established via the stable transfection of a myc-tagged KRS plasmid (7). The cells were maintained in RPMI-1640 medium (Welgene, Daegu, Korea) containing 10% FBS and antibiotics (Invitrogen, Grand Island, NY, USA).

Extract preparation and western blots. Colon cancer cells were washed with ice-cold phosphate-buffered saline (PBS) and lysed in a modified RIPA buffer (50 mM Tris-HCl, 150 mM NaCl, 1% NP-40 and 0.25% sodium deoxycholate) with a protease inhibitor cocktail (GenDepot, Barker, TX, USA). The extracts were centrifuged for 30 min at 15,000 X g at 4˚C, and then the lysates were collected from the supernatant. The protein amounts were normalized, before further immunoblotting with different antibodies. The primary antibodies used in the study were as follows and were against: α-tubulin (Sigma-Aldrich, St. Louis, MO, USA); pY^{416}c-Src, ERKs, and phospho-ERKs (Cell Signaling Technology, Danvers, MA, USA); paxillin and FAK (BD Biosciences, San Jose, CA, USA); pY^{397}FAK (Abcam, Cambridge, UK); c-Src, pY^{118}paxillin, pY^{577}FAK, pY^{861}FAK, pY^{925}FAK, Snail1, E-cadherin and β-catenin (Santa Cruz Biotechnology, Santa Cruz, CA, USA),

integrin α6 and β1 (Millipore, Billerica, MA, USA); and KRS (Atlas Antibodies, Stockholm, Sweden).

Normal culture or replating on ECM layer. Cells were kept in suspension or replated on serum (10%) or ECM (10 μg/ml laminin; BD Biosciences) -precoated dishes or coverglasses in the presence of 2% FBS for 1 h, before being analyzed for cell-serum or -laminin adhesion signaling by standard western blotting, as previously described (19). Pharmacological inhibitors were added to the culture media for 24 h or to replating media at the reseeding time. Cells were harvested for whole cell lysates, prior to immunoblottings for the indicated proteins, or imaging to monitor cell growth patterns. Confluent cells were wounded with a pipette tip and washed twice with PBS. After treatment with DMSO, U0126 (10 μM) LC Laboratories, Woburn, MA, USA), or YH16899 (10 μM) and incubation in a CO_2 incubator for 24 h, the marginal edges of the wounds were imaged (CKX41; Olympus, Tokyo, Japan).

Coimmunoprecipitations. HCT116 cells stably expressing myc-KRS WT in standard media containing 10% FBS in the presence of control vehicle or YH16899 treatment (10 μM) for 24 h were harvested for whole cell lysates, and lysates were immunoprecipitated overnight using anti-myc tagged antibody-coated agarose beads (Sigma-Aldrich). The immunoprecipitated proteins were boiled in 2X SDS-PAGE sample buffer before standard western blot analyses.

Indirect immunofluorescence. Cells reseeded in normal culture media- or laminin-precoated glass coverslips in 2% FBS-containing media for 2 or 24 h were stained for F-actin using phalloidin, or immunostained using antibodies against E-cadherin, β-catenin, N-cadherin, pERK, phospho-Tyr397, or phospho-Tyr118 paxillin, in addition to 4',6-diamidino-2-phenylindole (DAPI) staining for the nucleus. Immunofluorescence images were acquired on a fluorescence microscope (BX51TR; Olympus) or on a confocal laser scanning microscope with a Nikon Plan Apochromat 60x/1.4 N.A. oil objective (Nikon Eclipse Ti microscope; Nikon, Tokyo, Japan).

RT-PCR. Total RNA was extracted from cells under diverse experimental conditions, using TRIzol® (Invitrogen) according to the manufacturer's protocol. Total RNA (1 μg) was reverse transcribed using the amfiRivert Platinum cDNA Synthesis Master Mix (GenDepot). Primers were designed using Primer3 software as follows: human E-cadherin (*CDH1*) mRNA, forward 5'-TGCCCAGAAAATGAAAAAGG-3' and reverse 5'-GTGTATGTGGCAATGCGTTC-3'; human N-cadherin (*CDH2*) mRNA, forward 5'-ACAGTGGCCACCTACAAAGG-3' and reverse 5'-CCGAGATGGGGTTGATAATG-3'; human vimentin (*VIM*) mRNA, forward 5'-GAGAACTTTGCCGTTGAAGC-3' and reverse 5'-GCTTCCTGTAGGTGGCAATC-3'; human Twist (*TWIST*) mRNA, forward 5'-GGAGTCCGCAGTCTTACGAG-3' and reverse 5'-TCTGGAGGACCTGGTAGAGG-3'; human paxillin (*PXN*) mRNA, forward 5'-GAAATCAGCTGAGCCTTCAC-3' and reverse 5'-TTAGGCTTCTCTTTCGTCAGG-3', *Snail1*, forward 5'-GGTTCTTCTGCGCTACTGCT-3' and reverse 5'-TAGGGCTGCTGGAAGGTAAA-3'; *slug*, forward 5'-GGGGAGAAGCCTTTTTCTTG-3' and reverse 5'-TCTCATGTTTGTGCAGGAG-3';
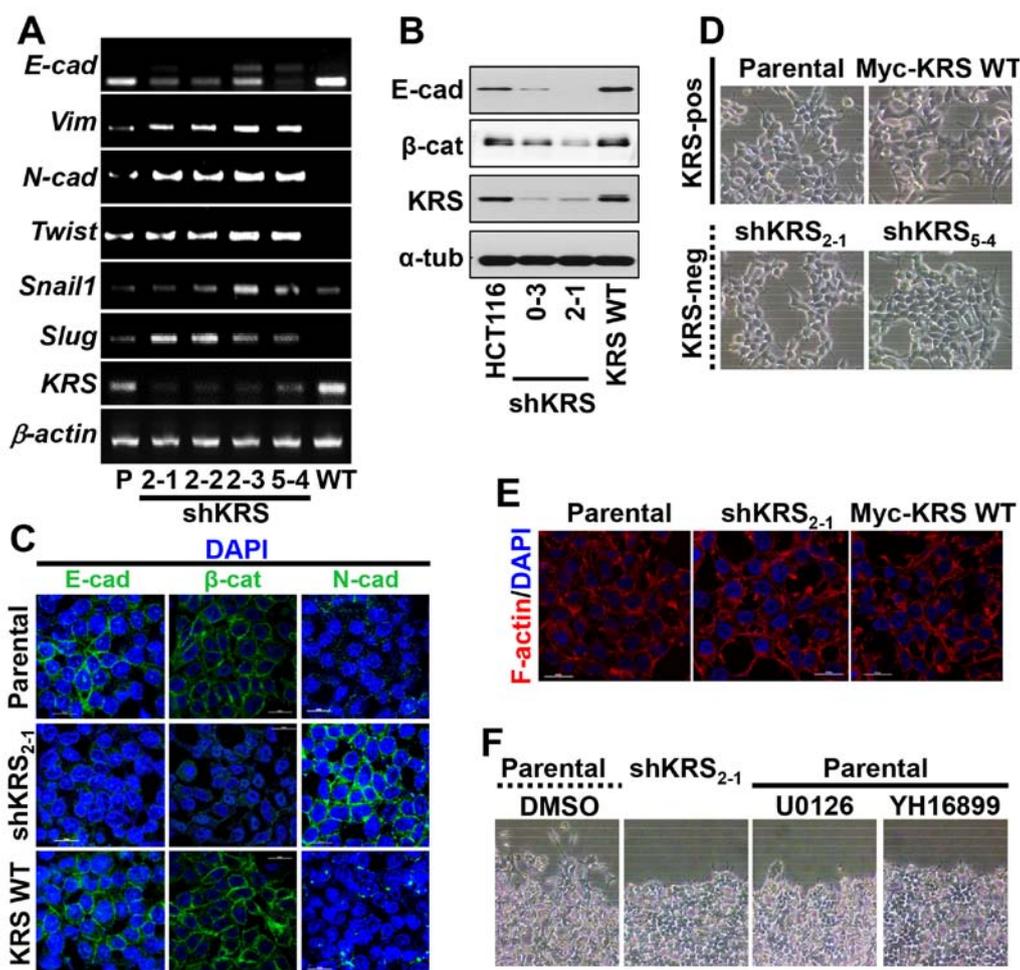
Figure 1. KRS downregulation causes incomplete EMT phenotypes. (A and B) RT-PCR (A) or standard western blots (B) for the indicated epithelial or mesenchymal markers were performed using KRS-expressing parental HCT116 (P), stably myc-KRS wild-type overexpressing HCT116 (KRS WT), and stably KRS-suppressed HCT116 cell clones (2-1, 2-2, 2-3 and 5-4 for shKRS) normally cultured in 10% FBS-containing condition. (C) Cells were cultured on coverglasses precoated with 10% FBS-containing culture media until confluence and then immunostained for the molecules along with DAPI costaining. (D and E) Subconfluent cells from the normal culture condition were imaged using a phase contrast microscope (D) or stained for F-actin before visualization using a fluorescent microscope (E). (F) Confluent cells grown under normal conditions were wounded, washed and treated with either DMSO or the indicated inhibitor. Fifteen hours later, cells near the wound edges were randomly imaged. Data shown represent at least three independent experiments.

human KRS (*KARS*) mRNA, forward 5'-CAATGCCCATGC CCCAGCCA-3' and reverse 5'-ACCCCACCCTTCCGGCG AAT-3'; human β-actin (*ACTB*) mRNA, forward 5'-TGAC GGGGTCACCCACACTGTGCCCATCTA-3' and reverse 5'-CTAGAAGCATTTGCGGTGGACGACGGAGGG-3'.

Statistical methods. The Student's t-test was performed for statistical comparisons of mean values to determine significance. A P-value <0.05 was considered to indicate a statistically significant result.

Results

KRS suppression caused an incomplete EMT phenotype. To explore the roles of KRS in metastatic potential, HCT116 colon cancer cells with endogenous, suppressed, or overexpressed KRS levels were first analyzed for EMT phenotypes. Using an RT-PCR approach, we found that KRS suppression decreased E-cadherin mRNA but increased the mRNAs of mesenchymal markers (Fig. 1A). Furthermore, these KRS suppression-dependent changes in EMT markers also applied

to protein levels; epithelial markers, including E-cadherin, were expressed at higher levels in KRS-expressing parental and KRS-overexpressing HCT116 cells, whereas KRS-suppressed cell clones had decreased E-cadherin and β-catenin expression (Fig. 1B). We then checked whether KRS downregulation could affect general protein translation and/or apoptosis, and there were no differences between KRS-expressing and KRS-suppressed cell clones (18). E-cadherin was not clearly observed in cell-cell junctions but N-cadherin was expressed in the junctions with minimal β-catenin when KRS was suppressed, as compared with KRS-expressing cells (Fig. 1C). Although the inverse relationship between KRS and epithelial marker expression might suggest the induction of EMT phenotypes, cell-cell contacts were not disrupted, presumably due to N-cadherin at the junctions after KRS suppression (Fig. 1C and D). Furthermore, cortical actin, but not stress fibers, was observed at the cell junctions independent of KRS expression (Fig. 1E). These observations may suggest that KRS suppression caused a partial EMT phenotype.
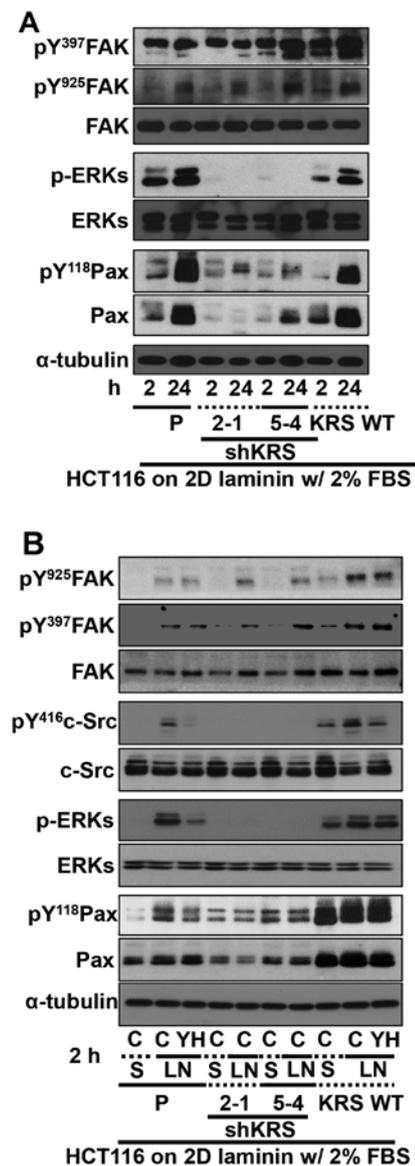
However, when wounds were made in confluent cells in normal 10% fetal bovine serum (FBS)-containing conditions,
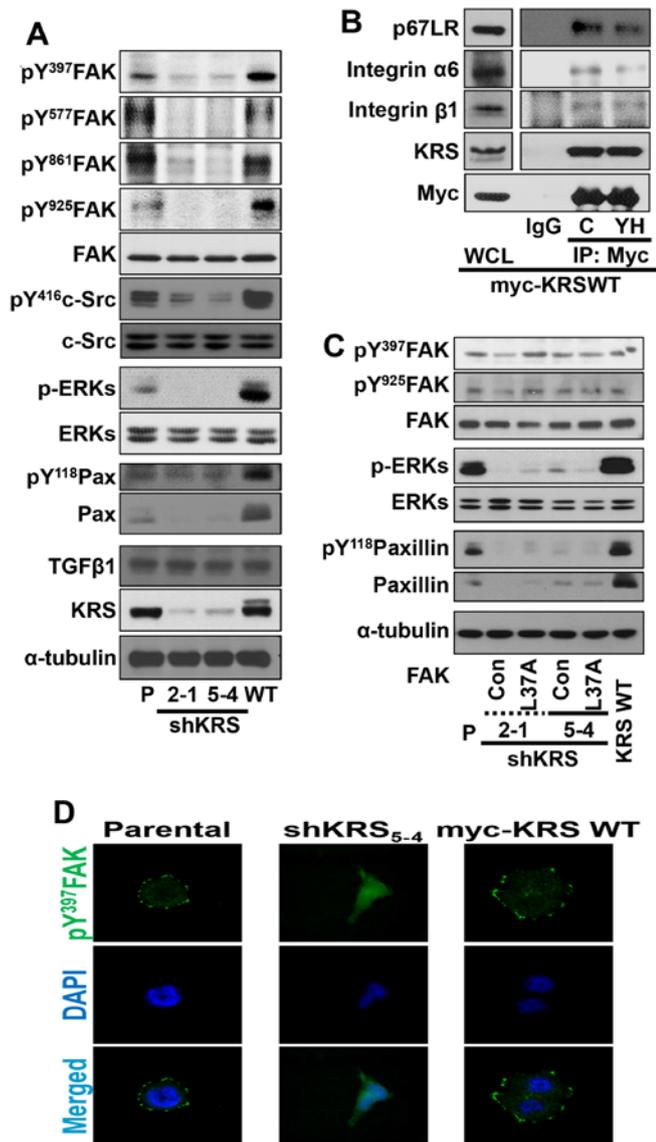
Figure 2. KRS suppression impairs cell adhesion-related signaling activities. (A) HCT116 cells with various KRS expression levels [parental P, KRS-suppressed 2-1 and 5-4 clones, and myc-KRS WT overexpressed (WT) cells] were analyzed for the indicated molecules via standard western blots. (B) Myc-KRS WT cells grown in normal culture conditions in the presence of vehicle DMSO (C) or YH16899 (YH) treatment were harvested for whole cell lysates (WCL) prior to immunoprecipitation using normal IgG (IgG) or anti-myc antibody and standard western blotting for the indicated molecules. (C) Stable HCT116 cells were transfected with control vector (Con) or L37A FAK mutant (L37A) for 48 h, before whole cell lysates preparation and standard western blots for the indicated molecules. (D) Cells replated overnight on coverglasses precoated with 10% FBS containing normal culture media were stained with DAPI for nuclei and immunostained for phospho-Tyr397 (pY397FAK), prior to saving the representative images using a fluorescent microscopy. Data shown represent three independent experiments.

Figure 3. KRS suppression impairs signaling activities mediated by cell-laminin adhesion. (A) Cells were reseeded onto laminin (LN)-precoated dishes under 2% FBS containing culture media for 2 or 24 h, prior to harvesting for standard western blots for the indicated molecules. (B) Cells were kept in suspension (S) or reseeded onto laminin (LN)-precoated dishes under 2% FBS containing culture media with DMSO (C) or YH16899 (YH) treatment for 2 h, prior to harvesting for standard western blots for the indicated molecules. Data shown represent three independent experiments.

the KRS-expressing parental cells exhibited clear outbound movement at the wound boundary, whereas KRS-suppressed cells did not (Fig. 1F). These KRS-dependent differential movements toward free spaces might be correlated with ineffective cell-substrate adhesion processes.

KRS suppression impaired cellular signaling and focal adhesion formation. We then examined the relationship between KRS expression and cell-ECM adhesion properties. First,

cells with various KRS expression levels were allowed to adhere under normal 10% FBS-containing culture conditions and were examined for adhesion-related signaling activities. Under normal 10% FBS-containing conditions, KRS suppression abolished the signaling activities of FAK, ERK, c-Src and paxillin, and the expression of paxillin, compared with KRS-expressing (or -overexpressing) cells (Fig. 2A). Integrins activate FAK, ERK and paxillin for diverse cellular functions in different cell systems (16). KRS is translocated to the plasma membrane when it associates and collaborates with p67LR (a non-integrin laminin receptor) in the presence of extracellular laminin, following Thr52-phosphorylation-dependent disassociation from cytosolic MSC (7). Integrin $\alpha6\beta1$ and $\alpha6\beta4$ respond to laminin and bind p67LR (9,10). Therefore, to
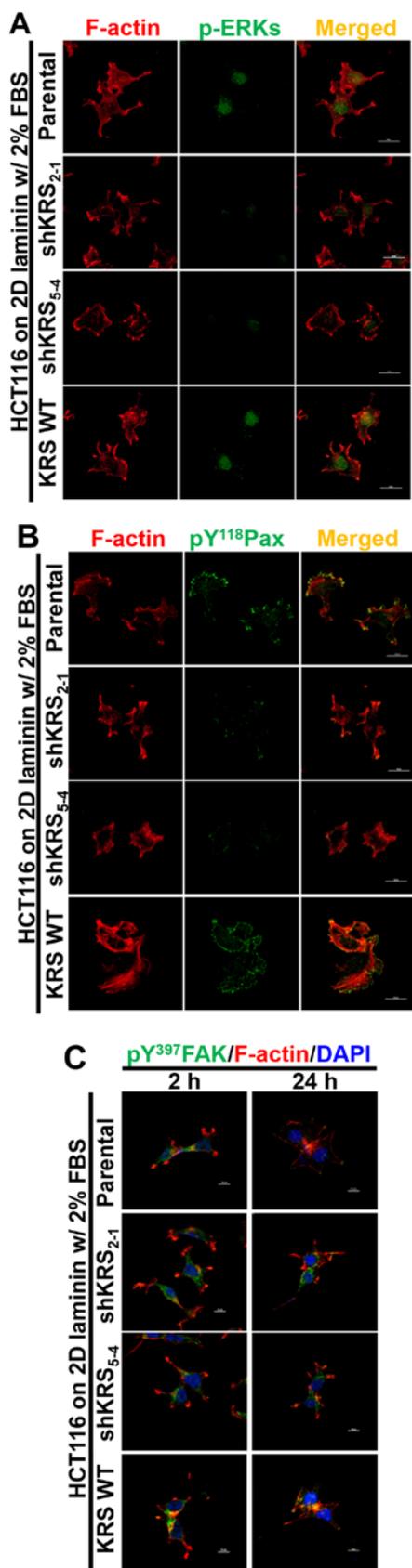
Figure 4. KRS suppression impairs formation of FAs with phospho-Tyr118 paxillin upon adhesion onto laminin. (A) Cells were reseeded onto laminin (LN)-precoated coverglasses under 2% FBS containing culture media for 2 (A-C) or 24 h (C), prior to staining for F-actin and immunostaining for pERKs (A), phospho-Tyr118 paxillin (pY118Paxillin, B), and phospho-Tyr397 FAK (pY397FAK, C). The representative images for each condition were selected among more than 10 random images and shown. Data shown represent three independent experiments.

determine whether the interaction between KRS, p67LR and integrins could be correlated to signaling activation for FAK, c-Src, ERKs and paxillin, we examined possible physical interactions between these proteins via a coimmunoprecipitation study. Myc-KRS in extracts prepared from normal 10% FBS-containing culture conditions co-precipitated integrins α6, and β1 and this interaction was blocked by the anti-KRS inhibitor (YH16899) treatment (Fig. 4C) (8).

In addition, stable transfection of L37A mutant FAK, which was shown to be active (20), into KRS-suppressed cells did not cause ERK activation, paxillin expression and Tyr118 phosphorylation (Fig. 2C), suggesting that KRS might regulate ERKs/paxillin signaling activity and expression independently of FAK activation. Furthermore, when phospho-Tyr397 FAK was imaged to visualize the focal adhesions (FAs), KRS-suppressed cells did not show FAs, whereas KRS-expressing cells showed well-formed FAs (Fig. 2D).

Cell adhesion-related signaling activities were then analyzed after the cells were kept in suspension or reseeded onto ECM-precoated culture dishes. Because KRS has been shown to translocate to the plasma membrane in a laminin-dependent manner (7), and because KRS suppression did not change laminin expression (18), we explored the biological significance of KRS expression after reseeding onto laminin-precoated plates under reduced (2%) serum-containing conditions. FAK phosphorylation depended on KRS expression in cases of parental or overexpressing cells, as reported previously showing that KRS overexpression in A549 cells further increased Tyr397 FAK phosphorylation upon being replated onto laminin-precoated dishes in the absence of serum (7). However, FAK phosphorylations in KRS-suppressed cells were somewhat comparable to that in parental HCT116 cells (Fig. 3A). KRS-dependent FAK activity in 10% serum-containing normal conditions (Fig. 2A) appeared different from HCT116 cells manipulated and newly adhered onto laminin under a lower serum conditions (Fig. 3A). The adhesion-dependent phosphorylation of FAK in KRS-expressing cells under these conditions was not affected by treatment with a KRS inhibitor YH16899 (Fig. 3B), which blocks the interaction between KRS and p67LR (Fig. 2B). However, the phosphorylation of ERK, paxillin and c-Src in KRS-expressing cells increased upon adhesion to laminin and was abolished by additional YH16899 treatment, whereas no effects were observed in KRS-suppressed cells (Fig. 3B).

We next examined the subcellular localization of phospho-ERK, phospho-Tyr118 paxillin, or phospho-Tyr397 FAK in cells with various KRS expression levels. When the cells were reseeded onto laminin-precoated coverglasses in 2% serum-containing media, KRS-positive (but not KRS-suppressed) cells showed obvious phospho-ERK staining in the nucleus (Fig. 4A). Phospho-Tyr118 paxillin was also clearly localized to FAs in a KRS-dependent manner (Fig. 4B). Notably, replating the cells onto laminin for 2 or 24 h resulted in comparable protrusions and spreading with comparable phospho-Tyr397 FAK levels, independent of KRS suppression (Fig. 4C). This suggested that FAK activity in response to laminin stimulation might not depend on KRS expression. However, ERK1/2 phosphorylation and paxillin expression and phosphorylation depended on KRS expression levels (Figs. 3 and 4).
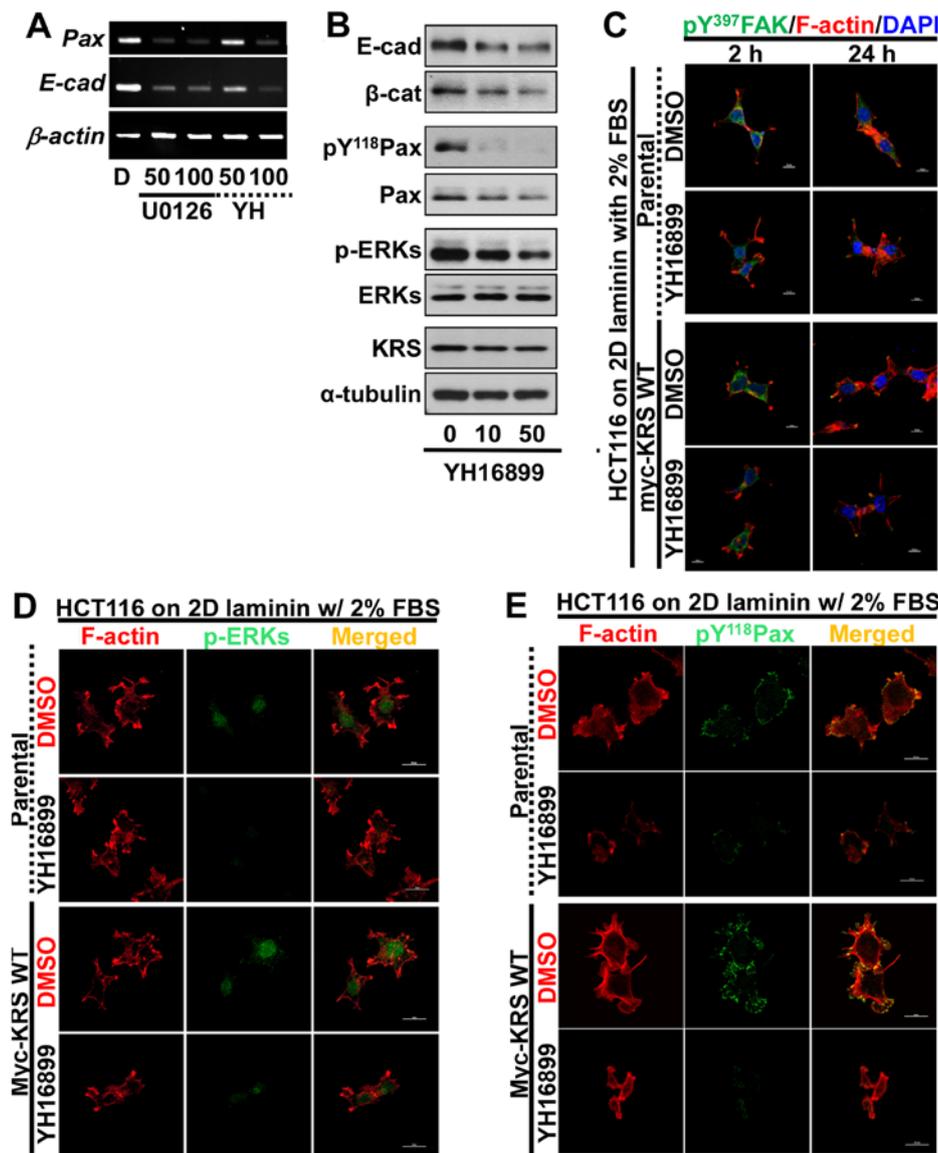
Figure 5. Anti-KRS reagent impairs formation of FAs with phospho-Tyr118 paxillin on cell-laminin adhesion. (A and B) HCT116 parental cells were treated with U0126 (50 or 100 μM) or YH16899 (50 or 100 μM) for 24 h, and the cells were processed for RT-PCR (A) or immunoblotting (B) for the indicated molecules. (C-E) Cells were reseeded onto laminin-precoated coverglasses for 2 h in the presence of DMSO (vehicle) or YH16899 (50 mM) treatment before immunostaining for pY^{397}FAK (C, green), pERK (D, green), or pY^{118}Paxillin (E, green) with phalloidin for F-actin (red) and DAPI for DNA (blue) costaining. Data represent three independent experiments.

Anti-KRS reagent abolishes KRS-dependent cell adhesion properties. We then explored whether KRS functioned on cell adhesion properties, using the anti-KRS reagent YH16899. Treating KRS-expressing cells with either U0126 or YH16899 decreased both paxillin and E-cadherin mRNA levels (Fig. 5A). Upon KRS inhibition by the YH16899 treatment, E-cadherin and β-catenin proteins also decreased, similarly to ERK and paxillin phosphorylations (Fig. 5B). However, YH16899 treatment of KRS-expressing cells did not result in changes in protrusion and spreading as visualized by Tyr397-phosphorylated FAK staining (Fig. 5C), but did result in decreased phospho-ERK (Fig. 5D) and phospho-Tyr118 paxillin (Fig. 5E), under the laminin-precoated conditions containing 2% FBS. These observations, therefore, suggested that the KRS-dependent cell adhesion activity could involve ERK and paxillin expression and activity.

Discussion

The present study suggests that KRS functions regulating cell-cell adhesion properties, leading to incomplete EMT, and cell-laminin adhesion-dependent signaling via an association with p67LR and integrin $\alpha6\beta1$ receptors. The roles of KRS in regulating cell-cell intercellular and cell-substrate adhesions by regulating E-cadherin expression, ERK activity, and paxillin expression and activity could promote cellular migration. During the KRS-dependent activation of the cell-substrate adhesion signaling pathway, FAK appeared unrelated to ERKs, paxillin activation and paxillin expression. This KRS/ERK/paxillin signaling axis may be a useful target against KRS-dependent cancer metastases.

KRS suppression modulated the epithelial-mesenchymal properties of cells; KRS suppression decreased the expression

of epithelial markers and concomitantly increased mesenchymal markers. However, how KRS causes these alterations should be explored further. Because mesenchymal markers increased upon KRS suppression, a possible global effect on protein translational capacity from the downregulated level of KRS was not the cause. Furthermore, KRS suppression did not cause cellular loss of adherence even after E-cadherin loss, presumably because mesenchymal N-cadherin replaced epithelial E-cadherin at the adherence junctions of KRS-suppressed cells. In addition to its roles in cell-cell contacts, KRS promoted cell-substrate adhesion signaling, which is important for myosin contractility-dependent adhesion strength and traction force (21) and for cellular contractility (22). Thus, KRS-mediated migration is involved in cell-substrate adhesion for contractility/traction force, so that cells can 'crawl out', even in the cases where cell-cell adhesion is well-formed via adherence junctions. The early phases of colon cancer metastasis are suggested to have adapted EMT-like de-differentiation at the invasive edges, in order to detach and migrate (23). HCT116 cells quite highly express E-cadherin and are categorized as the most epithelial-like cells in the EMT score among the diverse tumor cell types (24). Presumably, E-cadherin for cell-cell adhesions and cell-substrate adhesion in KRS-positive colon cancer cells may coordinately contribute to migration for efficient metastasis involving a transient EMT-like process. We observed that KRS-dependent migration did not depend solely on either epithelial or mesenchymal characteristics, because KRS-suppressed cells could not efficiently migrate, although they obviously lost E-cadherin and other epithelial markers. It was recently reported that circulating tumor cells (CTCs) originated from primary breast tumors consisting of cells with epithelial, mesenchymal, or both characteristics (25,26). Therefore, it is likely that KRS-expressing cells with certain epithelial characteristics have efficient migration.

KRS-suppressed cells did not move away from the boundaries of cell masses, therefore, the loss of epithelial markers was not sufficient for the outward movement. In addition to regulating cell-cell adhesion, KRS could also regulate cell-substrate adhesion as part of its pro-metastatic functions. KRS translocates from the cytosolic MSC to the plasma membrane after p38 MAPK-dependent Thr52 phosphorylation upon extracellular laminin stimulation, where it protects p67LR from ubiquitination-mediated degradation (7). Furthermore, the interaction between KRS and p67LR is critically involved in the lung metastasis of subcutaneously-injected mouse breast carcinoma 4T1 cells with KRS overexpression (8). The KRS/p67LR complex also included integrin $\alpha6\beta1$, allowing KRS-expressing cells to transduce intracellular signals under extracellular laminin-stimulated conditions. Moreover, extracellular laminin, but not collagen I, causes KRS translocation to the plasma membrane for binding to p67LR (7). Thus, it is likely that KRS may mediate ERK activation for pro-metastatic roles following specific adhesion-mediated signals from the extracellular microenvironment.

In the present study, KRS-dependent migration was shown to require ERK activity and paxillin expression and activity, leading to efficient FA formation; inactivating ERK or disrupting complex formation among KRS, p67LR and integrin $\alpha6\beta1$ blocked KRS-dependent effects. FAK, c-Src family kinases (SFKs), paxillin, and ERK1/2 are key

regulators of focal adhesion dynamics, especially during cell adhesion and migration (27,28). Cell-substrate adhesion activates ERK via a FAK Tyr925 phosphorylation-mediated signaling pathway from FAK to the Ras cascade upon integrin/ECM interaction (29). However, FAK activity was not relevant for the KRS-dependent ERK and paxillin activations and paxillin expression, because manipulation of FAK activity did not lead to ERK activity and paxillin expression/Tyr118 phosphorylation. FAK-independent ERK activation may occur during cell adhesion events. The association of integrins with caveolin-1 and Fyn (a SFK member) recruits Shc for ERK activation (30-32). Furthermore, the cell adhesion-dependent activation of CaMKII in vascular smooth muscle cells leads to ERK activation independent of FAK activity (33).

The signaling transduced from the KRS/p67LR/integrin $\alpha6\beta1$ complex to paxillin expression/phosphorylation via ERK activity could be targeted to treat colon cancer metastasis depending on KRS expression and functions.

Acknowledgements

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