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Inhibition of farnesyltransferase reduces angiogenesis by interrupting endothelial cell migration

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ABSTRACT

Inhibitors of farnesyltransferase (FTI) have been developed for cancer treatment for more than a decade. Aside from being a therapeutic target in tumor cells, little is known about the role of farnesyltransferase (FTase) in other physiological processes. In this study, we revealed the involvement of FTase in angiogenesis and showed that FTI inhibited angiogenesis by directly acting on endothelial cells. Inhibition of FTase interrupted cell migration in vitro and in vivo. In addition, we found that FTase was important for cell polarization, cell spreading and pseudopodia formation. We also found that FTase interacted with microtubule end binding protein 1 (EB1) and that this interaction was critical for the localization of EB1 to microtubule tips. Our findings thus offer novel insight into the functions of FTase in endothelial cells and provide valuable information for the use of FTI in cancer therapy.

1. Introduction

Farnesyltransferase (FTase) catalyzes the covalent attachment of a farnesyl group to the CAAX (C is cysteine, A is an aliphatic amino acid, and X is variable) motif at the carboxyl terminus of a protein [1,2]. Addition of this 15-carbon isoprenoid alters the properties of many important cellular proteins including nuclear lamins, kinetochore proteins CENP-E and CENP-F, as well as members of the Ras, Rho and RheB families [1,3,4]. Functional FTase is a dimer with its β subunit harboring the active site. Farnesylation has been shown to be critical for the proper localization of Ras and Rho to cell membrane by conferring them lipid-binding ability, which is prerequisite for their transforming power [4]. Linkage between farnesylation and activation of these oncogenic proteins hence prompted extensive studies focusing on the role of FTase in malignant cells, and attempts to use inhibitors of farnesyltransferase (FTI) for cancer treatment have made some progress in the last decade [1,3,5,6]. Despite the potency of FTI in reducing proliferation and inducing apoptosis of cancer cell lines, clinical trials showed mixed responses, warranting detailed characterization of FTase in alternative processes of tumor development and in other cell types within the tumor microenvironment [5–9]. Investigations outside of the cancer context are providing insights into the functions of FTase in more physiological events [4,10,11], but molecular mechanisms independent of oncogenic Ras are still largely unknown.

Angiogenesis is a multistep process by which the existing vascular network is elaborated by formation of new blood vessels from pre-existing ones [12–15]. Angiogenesis holds a fundamental role in development and also functions in other physiological settings. Pathological angiogenesis, however, contributes to tumor growth and metastasis by providing nutrients and oxygen and serving as ducts for tumor cells [12,14,15]. Failure of or insufficient angiogenesis results in ischemic chronic wounds, while excess angiogenesis leads to age-related macular degeneration, demonstrating the importance of balanced angiogenesis for homeostasis [12,15]. FTI has been shown to be anti-angiogenic in tumor xenograft assays, due to its role in diminishing the release of angiogenic factors by targeting Ras-related cascades in tumor cells [1,3,4]. Emerging evidence suggests a broader effect of FTI in angiogenesis, pointing to the involvement of FTase in multiple endothelial functions.

In both normal and pathological angiogenesis, vascular endothelial cells adopt conserved mechanisms to migrate in response to angiogenic signals [12,16]. During migration, endothelial cells maintain a polarized morphology and extend multiple protrusions in probing the surrounding area [13,16–18]. Accurate interpretation of migration cues reflects the angiogenic potential of endothelial cells, which results from the dynamics of cytoskeleton and requires the cooperation between the cytoskeleton and associated proteins [13,16,18]. Microtubule end-binding protein (EB1) is a well established microtubule associated protein (MAP) which controls microtubule dynamics and plays an important role in the formation of polarized morphology and the motility of cells

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[18,19]. FTase has also been found to bind directly to microtubules [20], but the effect of FTase on microtubule dynamics remains elusive.

In this study, we found that FTase was critically involved in angiogenesis by regulating endothelial cell migration. Detailed analysis revealed the importance of FTase in cell polarity, cell spreading and pseudopodia formation. Moreover, we found that FTase interacted with EB1 and that inhibition of FTase interrupted the proper localization of EB1 to microtubule tips. Our results thus demonstrate the role of FTase in non-transforming cells and offer novel insight into the pharmacology of FTI in cancer treatment.

2. Materials and methods

2.1. Materials

The farnesyltransferase inhibitor SCH66336 and an inactive isomer of SCH66337, were from Schering-Plough (NY, USA). Matrigel was purchased from BD Biosciences (NY, USA). Antibodies against α-tubulin, γ-tubulin, actin and EB1 were obtained from Sigma–Aldrich (MO, USA). Small interfering RNAs (siRNA) targeting FTase were synthesized from Ribobio (Guangzhou, China).

2.2. Cell culture and transfection

Human umbilical vascular endothelial cells (HUVECs) were routinely maintained using RPMI1640 with 10% fetal bovine serum, in standard humidified cell incubator with 5% CO_2 at 37 °C. Cell transfection was performed with Lipfectamine 2000 reagent (Invitrogen, CA, USA).

2.3. RT-PCR

Total RNA was extracted from cells by TRIzol Reagent from Invitrogen (CA, USA). 2 μg RNA was used from each sample to perform cDNA synthesis with Reverse Transcriptase M-MLV from Promega (WI, USA). RT-PCR was then carried out using same amount of cDNA under thermal conditions as follows: 94 °C 5 min, 25 cycles of 94 °C 30 s, 56 °C 30 s and 72 °C 45 s, and 72 °C 5 min. PCR product was resolved by agrose gel and visualized under UV illumination. GAPDH was used as internal control.

2.4. Wound healing assay

Wound healing assay was performed as described before [13]. After treatment with FTI or transfection with siRNAs, HUVECs were allowed to reach confluence on 24-well plate. A pipette tip was used to create wound, with cell debris removed by several washes of PBS. Culture medium was added afterwards and the extent of subsequent wound healing recorded by imaging in 24 h. This assay was repeated 3 times and the percentage of wound closure was analyzed with ImageJ (NIH).

2.5. Tube formation assay and spheroid sprouting assay

Tube formation assay was performed as previously described [13,18]. HUVECs treated with FTI or transfected with siRNAs were resuspended and plated on 6-well plate precoated with matrigel. This assay was repeated 3 times. Images were taken in 6 and 24 h and analyzed with ImageJ. To make spheroids, HUVECs were seeded into round-bottom 96-well plate with culture medium containing 0.2% carboxymethylcellulose for 8 h. Spheroids of HUVECs were embedded into collagen afterwards and induced to form sprouts under normal culture condition. This assay was repeated 3 times. Images were taken and sprouts analyzed with ImageJ (NIH).

2.6. Matrigel plug assay

300 μL matrigel mixed with FTI or PBS was injected subcutaneously into 4–5 weeks BALB/c mice, and 3 mice were used in each group. The matrigel plug was recovered in 14 days and processed in accordance with standard histological procedures. Haematoxylin eosin (H&E) staining was performed to reveal the extent of cell migration inside matrigel. The use of mice was approved by the Animal Care and Use Committee of Nankai University.

2.7. Cell polarization assay

Monolayer of HUVECs was scratched with pipette tip to stimulate directed cell migration, with immunostaining of α-tubulin and γ-tubulin performed to visualize the organization of microtubules and the position of microtubule organizing center (MTOC). 4,6-Diamidino-2-phenylindole (DAPI) was used for DNA staining. Border cells with MTOC situated between the nucleus and the leading edge were taken as polarized.

2.8. Cell spreading assay

HUVECs treated with FTI or transfected with siRNAs were resuspended from dishes and re-seeded on coverslips with normal medium. Cells were fixed after 30 min with 4% paraformaldehyde and stained for actin to better visualize pseudopodia. This assay was repeated 3 times.

2.9. Immunofluorescent microscopy

Cells were fixed with 4% paraformaldehyde on coverslips and blocked with 2% bovine serum albumin. Primary and secondary antibodies were diluted and applied according to the instructions as well as previous knowledge. DAPI was used for DNA staining at the end of incubation and coverslips mounted with 90% glycerol. Images were taken with a fluorescence microscope and analyzed with ImageJ.

2.10. GST pull-down and western blotting

GST pull-down was performed as previously described [21,22]. Purified GST-EB1 was immobilized on glutathione Sepharose beads, which were incubated with lysate from control cells or cells overexpressing FTβ at 4 °C for 2 h. The beads were washed afterwards, proteins released and resolved by SDS-PAGE. Resolved proteins were then transferred onto PVDF membranes by electricity in accordance with standard protocols. After blocking with fat free milk, membranes were probed with primary and horseradish peroxidase-conjugated secondary antibodies. Band visualization was achieved by applying chemiluminescent substrate (Pierce, IL, USA).

2.11. Statistics

Analysis of differences between groups was performed using Student's *t*-test. Graphs were drawn and P values calculated using GraphPad Prism 5.

3. Results

3.1. FTase is essential for angiogenesis

The effect of FTase on angiogenesis was studied using HUVECs. Two different siRNAs were utilized, which reduced the transcription level of FTase to 38.6% and 19.5% of control group, respectively (Fig. 1A and B). When plated on matrigel, HUVECs began to form

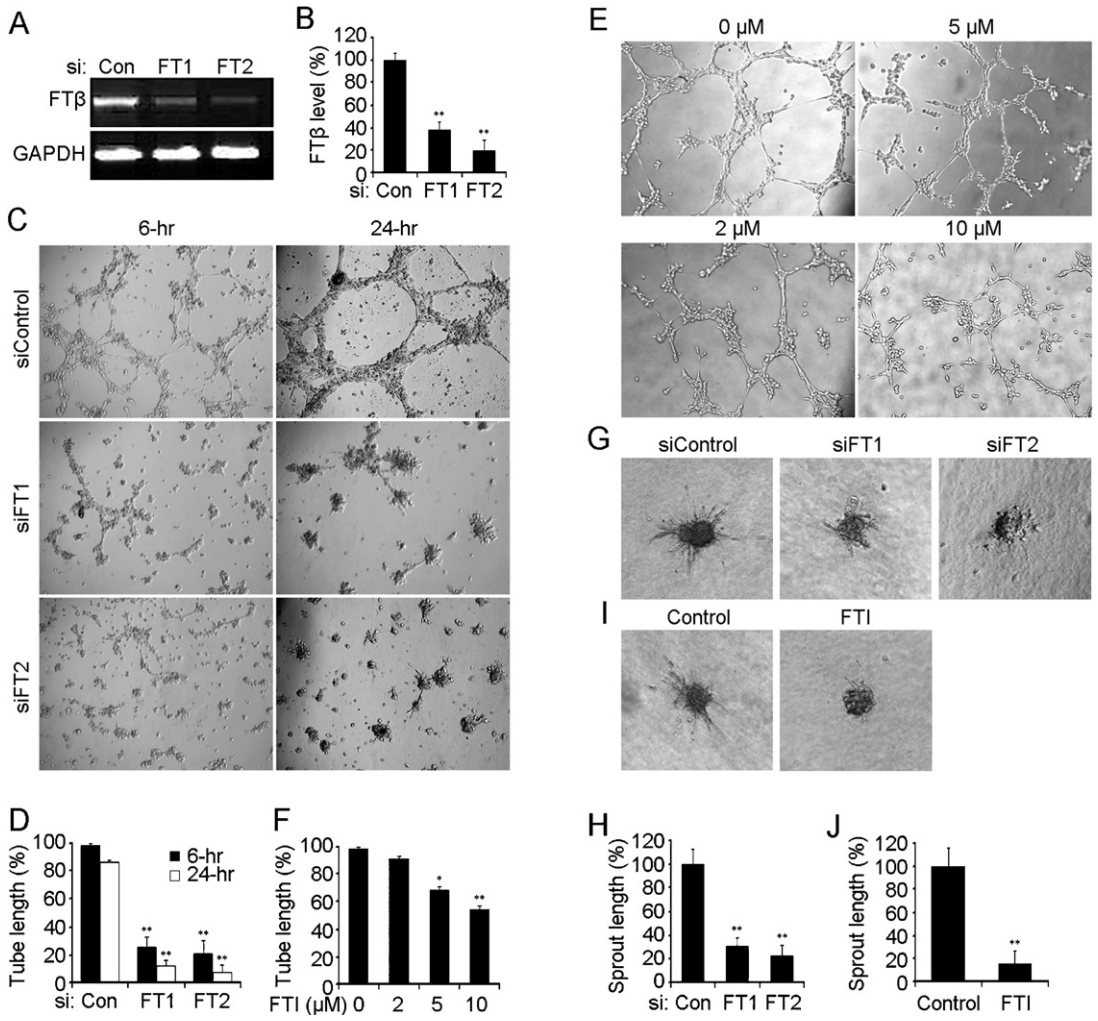

Fig. 1. FTase is essential for angiogenesis. (A) RT-PCR was performed to evaluate the efficiency of siRNAs in reducing the transcription levels of FTβ (the catalytic subunit of FTase). GAPDH was used as internal control. (B) Quantification of the efficiency of siRNAs. FTβ levels were normalized to the levels of GAPDH, with control group being 100%. (C) HUVECs transfected with siFTs or siControl were plated on matrigel to allow for tube formation, with images taken in 6 h and 24 h. (D) Cumulative tube length in panel C was quantified and analyzed, with tube length in siControl group in 6 h being 100%. (E) Tube formation assay was performed with different concentrations of FTI from 0 to 10 μM, and images taken in 6 h. (F) Tube formation assay was performed as in E, and cumulative tube length was calculated. Total tube length in control group was set as 100%. (G) Spheroids of HUVECs after transfection with siControl and siFTs were embedded into collagen and induced to form sprouts. Images of sprouting spheroids were taken afterwards. (H) Cumulative length of sprouts were quantified and analyzed, with control group being 100%. (I) Spheroids of HUVECs with or without FTI treatment were induced to sprout in collagen and images were taken afterwards. (J) Experiments were performed as in I, and the total length of sprouts were calculated and analyzed. Values, means of three independent experiments; bars, SD; $^{*}P < 0.05$; $^{**}P < 0.01$.

tube-like structure, which were easily recognized after 6 h, and became more evident after 24 h (Fig. 1C). The accumulated tube length at a given time was measured as an index of angiogenesis (Fig. 1D). After transfection with siFT1 or siFT2, HUVECs displayed significantly reduced ability to form tube-like structure at both time points, as demonstrated by accumulated tube length (Fig. 1C and D). Such phenomenon was reproduced when FTI was applied to specifically inhibit the activity of FTase (Fig. 1E), with the angiogenesis indices decreasing at a dose-dependent manner from 2 μM to 10 μM (Fig. 1F).

To further corroborate this finding, we performed another in vitro angiogenesis assay, in which HUVECs aggregated as spheroids were seeded into collagen to mimic angiogenesis in a 3-dimensional environment [13]. In control group, sprouts from spheroids were obvious after a short period of incubation (Fig. 1G), indicating active angiogenesis. However, when transfected with siFT1 or siFT2, HUVECs became quiescent and sprouts were almost absent (Fig. 1G and H). Similarly, after treatment with FTI, there were significantly less sprouts from spheroids (Fig. 1I and J). Taken together, we demonstrated that FTase was essential for angiogenesis.

3.2. Inhibition of FTase prevents cell migration

We went on to study the effect of FTase on cell migration. Monolayer of HUVECs was scratched with a pipette tip, and the subsequent closure of wounded area was documented to evaluate the capacity of cell migration. As seen in Fig. 2A, the wounded area in control group was fully recovered after 24 h as a consequence of directed cell migration. In contrast, in both siFT1 and siFT2 groups the migration of HUVECs were retarded (Fig. 2A), leaving 61.2% and 62.9% wounded areas unoccupied respectively after the same time (Fig. 2B). FTI also showed potent efficiency in preventing cell migration, reducing wound closure to 24% of control group (Fig. 2C and D). Cytotoxicity of FTI was measured prior to this experiment, and no significant difference in cell number was found under the experimental settings (data not shown). Such influence on cell migration was lost when HUVECs were treated with SCH66337, an agent that had structural similarity to FTI but no effect on FTase (Fig. 2C and D), confirming the specificity of inhibition.

The significance of FTase in cell migration was also demonstrated in vivo. Matrigel mixed with FTI or PBS was injected

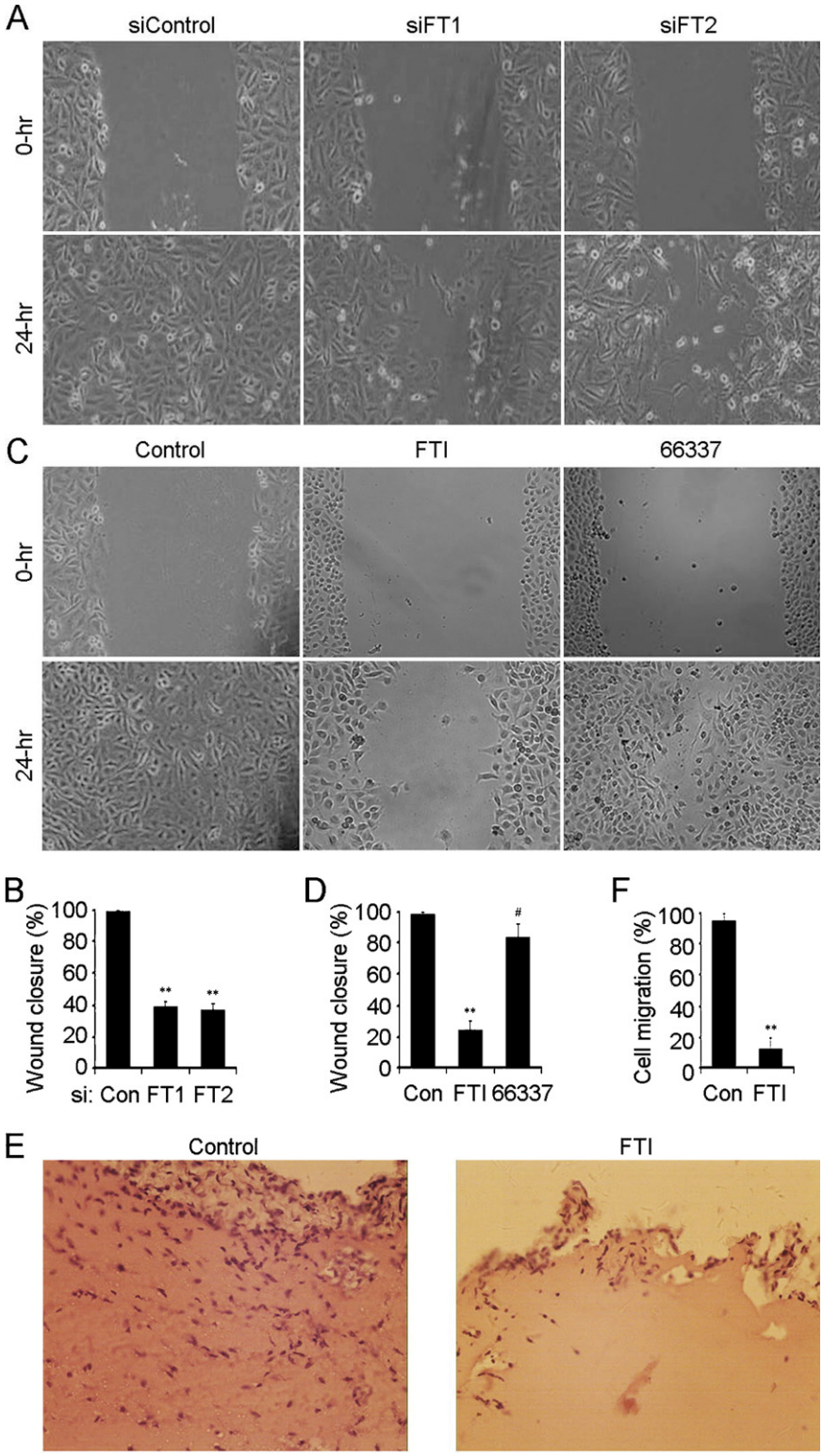

Fig. 2. Inhibition of FTase prevents cell migration. (A) HUVECs transfected with siControl or siFTs were cultured to reach confluence, which were scratched and allowed to heal. Images of the same area immediately after wounding and in 24 h were taken for analysis. (B) Percentage of wound closure were quantified and analyzed, with results from control group being 100%. (C) HUVECs treated with DMSO, FTI or SCH66337 were grown into monolayers and then scratched, with images of the wounded areas taken at different time points. (D) Wound closure were quantified and analyzed between different groups, with percentage from control group being 100%. (E) 300 μL matrigel mixed with PBS or FTI were injected subcutaneously into mice, which were recovered in 2 weeks and stained to visualize invading cells. (F) Cell migration into matrigel was quantified and analyzed, with total cell number in control group being 100%. Values, means of three independent experiments; bars, SD; $^{\#}P > 0.1$; $^{**}P < 0.01$.

subcutaneously into mice, which was harvested after two weeks, sectioned and stained with H&E to visualize migrated cells. Despite the strong ability of matrigel in inducing cell migration, as can be seen in Fig. 2E, FTI effectively blocked the influx of cells, reducing the extent of cell migration to 14% of control group (Fig. 2E and F). This was consistent with our in vitro data and further demonstrated the potency of FTI in reducing cell migration.

3.3. Inhibition of FTase disturbs cell polarization

As a preparation for migration, cells first establish polarity in response to stimuli, resulting in asymmetric organization of their inner structures [12,16,23]. This is a crucial step in achieving directionality. Cell polarity must also be maintained during the course of migration to ensure the fidelity of response to migration cues [24]. Defects in polarization often lead to the failure or inefficiency of

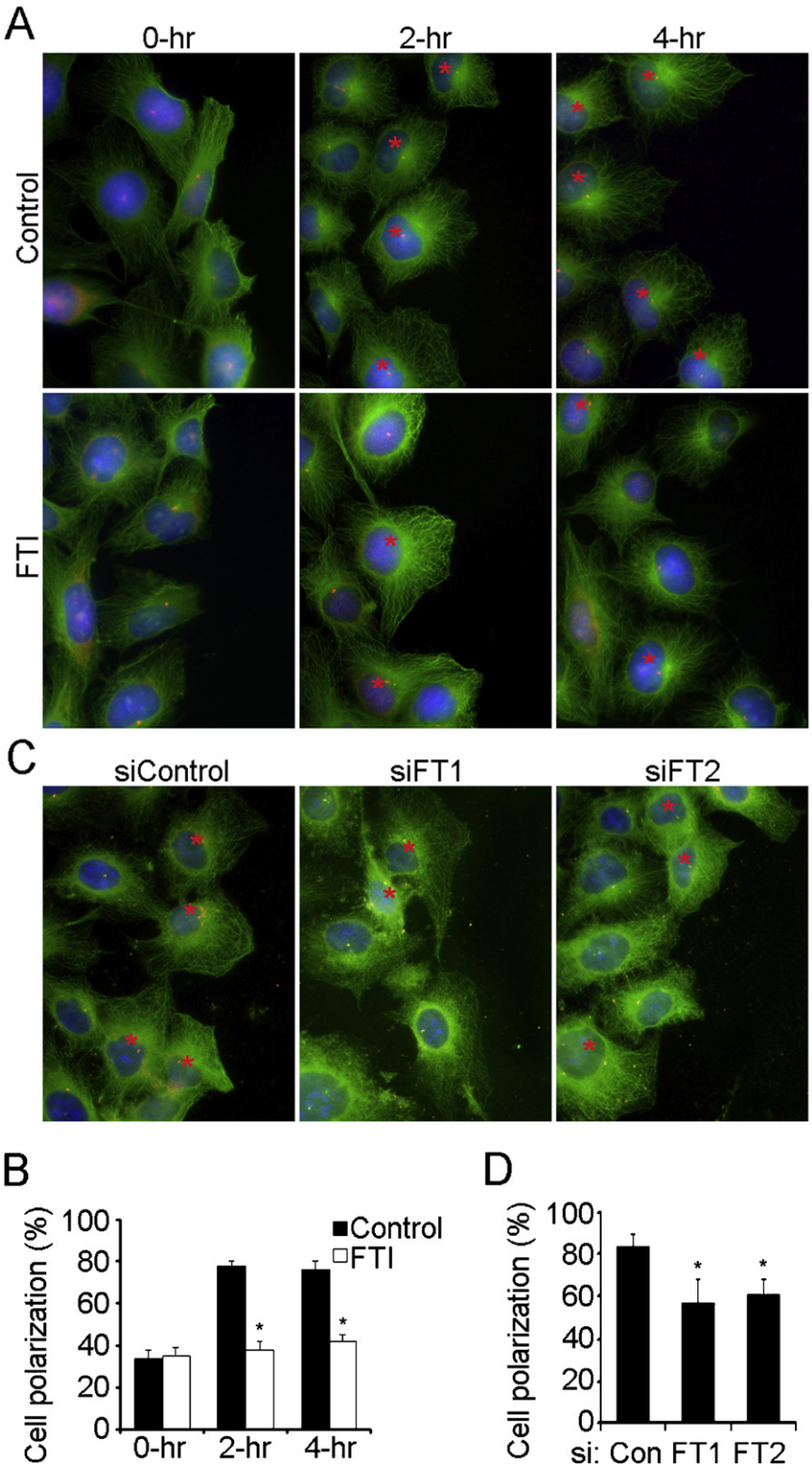

Fig. 3. Inhibition of FTase disturbs cell polarization. (A) HUVECs were stained for microtubule (green), centrosome (red) and DNA (blue) after scratching to show their arrangement of inner structures. Cells were treated with DMSO or FTI, and images were taken in 2 h and 4 h, respectively. *, polarized cell. (B) Analysis of cell polarization at different time points in A. Cells at the border of wound with centrosome facing the migration direction were viewed as polarized. (C) HUVECs transfected with siControl or siFTs were used to perform wound healing assays, with immunostaining to visualize the position of microtubule (green), centrosomes (red) and DNA (blue). *, polarized cell. (D) Experiments were performed as in C, and cell polarization analyzed by counting cells with polarized morphology to total number of cells at the foremost of migration. Values, means of three independent experiments; bars, SD; $^{*}P < 0.05$.

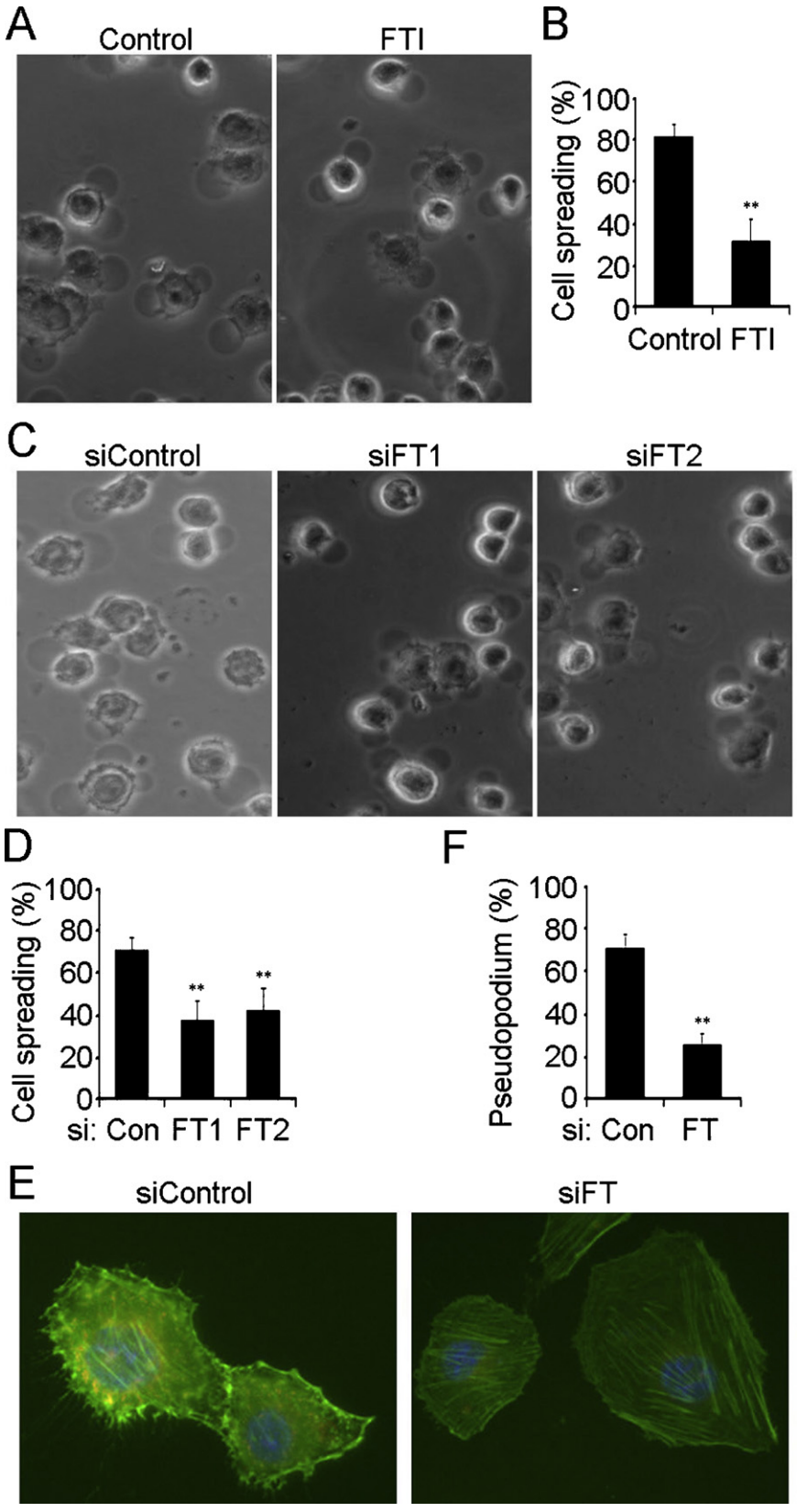

Fig. 4. FTase promotes cell spreading and pseudopodium formation. (A) After treatment with DMSO or FTI, HUVECs were collected and re-seeded, and the spreading of cells in 30 min was imaged. (B) Analysis of cell spreading in A. Cells with flat borders were considered as spreading, as opposed to rounded cells. (C) HUVECs transfected with siControl or siFTs were collected and re-seeded, and images were taken in 30 min. (D) Experiments were performed as in C, and percentage of spreading cells was counted and analyzed. (E) Experiments were performed as in C, with immunostaining for actin to better visualize pseudopodia. (F) Numbers of pseudopodia in E were counted and analyzed. Values, means of three independent experiments; bars, SD; $^{**}P < 0.01$.

cell migration [13,25]. One distinct feature of polarized endothelial cells is the placement of microtubule organizing center (MTOC) between leading edge and nucleus, which was adopted as a hallmark of cell polarization [13,16,18]. To evaluate the extent of cell polarization, we stained the microtubule cytoskeleton and MTOC of wounded HUVECs monolayer, and calculated the percentage of polarized cells at the forefront of migration sheet. Cells with MTOC facing the wounded area were defined as polarized, which constituted 35% of all cells immediately after wounding (Fig. 3A and B). The percentage of polarized cells increased to 78% 2 h after incubation, which was sustained after 4 h, where the majority of cells had embarked on directed migration to unoccupied area (Fig. 3A and B). However, starting with a comparable level of cell polarization with control group, HUVECs treated with FTI failed to properly orient themselves to the direction of migration afterwards, and the percentage of polarized cells was only slightly above the baseline level even after 4 h (Fig. 3A and B). Similarly, when the expression of FTase was decreased by siRNAs, the defect in cell polarization became evident, as could be seen in both Fig. 3C and D. Collectively, we showed the importance of FTase in cell polarization, which accounted in part for the function of FTase in regulating cell migration.

3.4. FTase promotes cell spreading and pseudopodia formation

Aside from its involvement in the initial step of cell migration, we studied the action of FTase on other aspects of cell motility [13,17,26]. In doing so, we digested and re-plated HUVECs to examine their response to a new environment. HUVECs spread readily on plate soon after being seeded, as characterized by flat edges around the cell body. The percentage of spreading cells was 81.6% after 30 min, showing a rapid response (Fig. 4A and B). In contrast, after treatment with FTI, cells were slow in adjusting their morphologies and the percentage of spreading cells was 32.3% after 30 min, with the majority of cells keeping round shapes (Fig. 4A and B). HUVECs transfected with siControl displayed no defect in spreading, whereas when transfected with siFT1 or siFT2 the percentage of spreading cells dropped to less than 42%, showing clearly attenuated dynamics (Fig. 4A and B).

Another feature of migrating cells is the presence of pseudopodia, a cytoplasmic structure commonly used to sense the gradient of migrating signals [12,16]. Newly settled HUVECs often form a large amount of pseudopodia before deciding on a migrating direction [13,26], which can be better visualized by immunostaining of actin, because of the abundance of actin bundles present in pseudopodia. As demonstrated in Fig. 4E, immediately after settlement there emerged several pseudopodia at the edges of cells, which were barely seen after cells were transfected with siFTs (Fig. 4F). Lack of this cytoplasmic projection following the down regulation of FTase indicated the significance of FTase in regulating the dynamics of membrane structures, which was consistent with its role in affecting cell migration.

3.5. Inhibition of FTase disrupts EB1 localization

FTI had been shown to induce extensive morphological changes in transformed cells, which was linked to its ability to disrupt

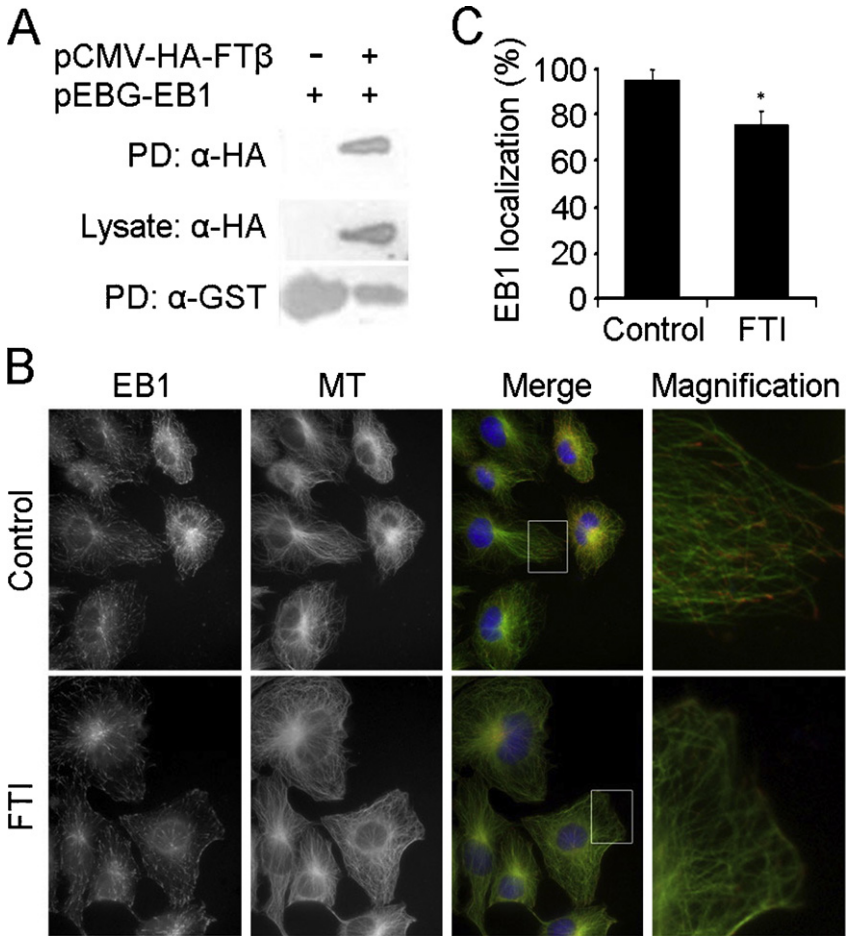

Fig. 5. Inhibition of FTase disrupts EB1 localization. (A) Cell lysates from 293 T cells transfected with or without pCMV-HA-FTβ (the catalytic subunit of FTase) were incubated with beads linked with GST-EB1 to detect the interaction between FTase and EB1. (B) HUVECs treated with DMSO or FTI were stained for EB1 and microtubule to show the localization of EB1 after inhibition of FTase. Leading edge of cell was magnified to better visualize the localization of EB1 at microtubule tips. (C) Analysis of EB1 localization in B. EB1 with localization at the end of microtubule filament was considered as positive. Values, means of three independent experiments; bars, SD; $^{*}P < 0.05$.

microtubule networks [27]. Moreover, FTase could bind directly to microtubules, which was important for its activation [20]. Coupled with our data showing an implication of FTase at the leading edge of migrating cells, we proposed the existence of more microtubule-associated proteins (MAP) that could be influenced by FTase, which most likely resided at the tips of microtubules to participate in the regulation of the dynamics of microtubules, as well as the interaction with the actin cytoskeleton and cell membrane. We tested this possibility by searching for novel partners of FTase. Of note, we found that EB1 interacted with FTase in vivo, which was confirmed by GST pull-down assay (Fig. 5A). Also, treatment with FTI significantly blocked the localization of EB1 to the tip of microtubules, which could be seen more clearly at the last panel of Fig. 5B and the statistical analysis (Fig. 5C).

3.6. Model

We propose a model for the function of FTase in endothelial cells. Through interacting with EB1, FTase localizes to the plus end of microtubules, where the substrates of FTase are translocated into cell membrane after farnesylation. FTase also helps to ensure the proper localization of EB1. After treatment with FTI or siFTs, the catalyzing ability of FTase is inhibited, leading to insufficient farnesylation of substrates as well as their absence in the cell membrane. Inhibition of FTase also interrupts the functions of EB1 by blocking its localization to microtubule tips, resulting in rigid response of microtubules and subsequently causes defects in cell migration and angiogenesis (Fig. 6).

4. Discussion

Efforts in developing FTI for cancer treatment in the last 20 years result mainly from the finding that the transforming ability of oncogenic Ras can be diminished by targeting FTase [1,3,4]. Indeed, FTI shows potent antitumor property through in vitro assays by inhibiting proliferation and inducing apoptosis of malignant cells, and it appears that this antitumor property is achieved by interrupting Ras related pathways [1,3]. In tumor xenograft models FTI also performs well, but outcomes from clinical trials are far from satisfying [3]. In particular, FTI shows negative results for pancreatic cancer, where 90% of this type of cancer harbors permanently active Ras [3]. In other cases, however, the positive result seems not associated with the ability of FTI to target Ras [3,9]. It is reasonable to assume that other interacting partners of FTase potentially account for this discrepancy, which calls for further studies about the functions of FTase in a more general context.

In comparison with cancer cells, the role of FTase in non-transforming cells remains painfully elusive. Considering the critical role of endothelial cells in tumor progression and metastasis, investigations into the functions of FTase in endothelial cells may help to explain the antitumor efficacy of FTI in different settings [8,10]. We demonstrated the direct involvement of FTase in affecting endothelial cell behaviors in environments mimicking the initial stages of angiogenesis in vitro, and FTI was also shown to be effective in influencing the proper response of endothelial cells under angiogenic stimulations. More experiments are still needed to examine the effects of FTI in vivo where actual blood vessels are formed with the presence of various types of cells. However, our data are helpful in expanding the pharmaceutical targets of FTI, which offered new perspectives when thinking about FTI treatment [14,15]. Also, the varying efficacy of FTI for tumors with different metastatic abilities may be better explained by taking endothelial cells into consideration [7,9,28]. Moreover, considering the involvement of FTase in angiogenesis and the availability of the large repertoire of FTI, patients suffering from diseases related to angiogenesis could benefit from the findings of this study.

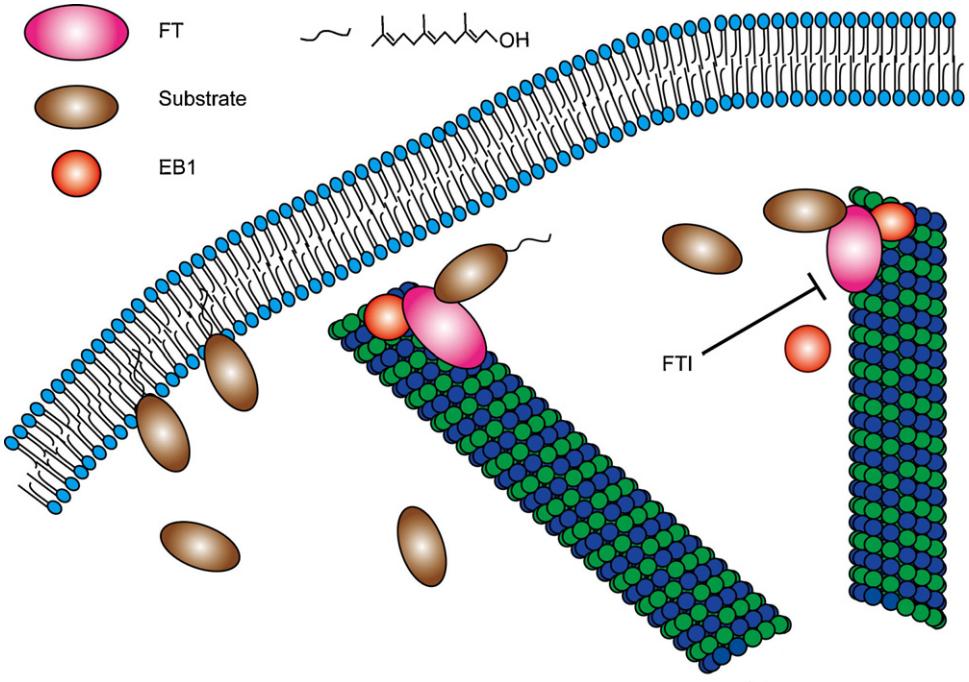

Fig. 6. Model of the function of FTase in endothelial cells. FTase is recruited to the distal end of microtubules via its interaction with EB1, where the addition of a farnesyl group to its substrates is followed by the insertion of the farnesylated protein into cell membrane. FTase also helps to localize EB1 to microtubule ends, possibly through its interaction with other proteins or by its direct interaction with microtubules. After FTI treatment or transfection with siFTs, the catalytic ability of FTase is inhibited, leading to insufficient activation of its substrates. Inhibition of FTase also disrupts the localization of EB1 to microtubule ends, thereby interfering with the dynamics of microtubules and causes inadequacy in cell polarization, cell spreading and pseudopodium formation, ultimately leading to defects in cell migration and angiogenesis. Cell membrane, microtubules and proteins are not drawn to scale.

The effects of FTI on endothelial functions are reported earlier, and FTI-induced defective endothelial cell migration in response to VEGF has been documented [10]. Delay in wound healing has been found in 6-month-old FTase deficient mice, but with no investigation at cellular level [11]. After FTase perturbation, defects in directed endothelial migration following artificial scratching was shown in vitro, and we report here that FTase contributes to cell migration by participating in the establishment and maintenance of cell polarity. Without FTase, endothelial cells become defective in assembling their inner structures in a polarized manner. In addition, endothelial cell dynamics are inhibited, resulting in retarded response to signals, as reflected by defective cell spreading and pseudopodium formation. Absence of pseudopodia insulates cells from their environment and intensified the delay in migration.

The interaction between FTase and EB1 opens avenues for further characterization of signaling events [15,29,30]. The activation of FTase has been found to be dependent on its binding to microtubules, which is also important for the proper functions of HDAC6, a binding partner of FTase [20]. Likewise, inhibition of FTase affected the localization of EB1 to microtubule tips, thus interrupting the normal functions of EB1 in recruiting other microtubule associated proteins, which results in the dysregulation of microtubule dynamics [21,22,30]. On the other hand, proper interaction between FTase and EB1 could facilitate the translocation of farnesylated substrates to cell membrane, adding to the mode of action of FTase [31]. In addition, the binding status of FTase itself could be adjusted following the interaction with another tightly regulated protein. Finally, in light of the close relationship between FTase and the microtubule cytoskeleton, development of combined therapies with agents targeting microtubules and FTI are merited.

Acknowledgments

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Contributors: Guoyuan Peng, Yuan Ren and Xiaodong Sun contributed study conception and design. Jun Zhou and Dengwen Li made acquisition, analysis and interpretation of data. Yuan Ren and Dengwen Li wrote and revised the work for intellectual content and context. Dengwen Li made final approval and overall responsibility for the published work. Dengwen Li assumes overall responsibility for the manuscript, and all authors share equally in certifying the originality of the work and the integrity of the report.

References

[1] Mazieres J, Pradines A, Favre G. Perspectives on farnesyl transferase inhibitors in cancer therapy. Cancer Lett 2004;206:159–67.
[2] Galjart N. Plus-end-tracking proteins and their interactions at microtubule ends. Curr Biol 2010;20:R528–37.
[3] Berndt N, Hamilton AD, Sebti SM. Targeting protein prenylation for cancer therapy. Nat Rev Cancer 2011;11:775–91.
[4] Sebti SM. Protein farnesylation: implications for normal physiology, malignant transformation, and cancer therapy. Cancer Cell 2005;7:297–300.
[5] Sharma V, Shaheen SS, Dixit D, Sen E. Farnesyltransferase inhibitor manumycin targets IL1beta-Ras-HIF-1alpha axis in tumor cells of diverse origin. Inflammation, in press, doi:10.1007/s10753-011-9340-6.
[6] Li T, Christos PJ, Sparano JA, Hershman DL, Hoschander S, O'Brien K, et al. Phase II trial of the farnesyltransferase inhibitor tipifarnib plus fulvestrant in hormone receptor-positive metastatic breast cancer: New York Cancer Consortium Trial P6205. Ann Oncol 2009;20:642–7.
[7] Balasis ME, Forinash KD, Chen YA, Fulp WJ, Coppola D, Hamilton AD, et al. Combination of farnesyltransferase and Akt inhibitors is synergistic in breast cancer cells and causes significant breast tumor regression in ErbB2 transgenic mice. Clin Cancer Res 2011;17:2852–62.
[8] Hong DS, Cabanillas ME, Wheler J, Naing A, Tsimberidou AM, Ye L, et al. Inhibition of the Ras/Raf/MEK/ERK and RET kinase pathways with the combination of the multikinase inhibitor sorafenib and the farnesyltransferase inhibitor tipifarnib in medullary and differentiated thyroid malignancies. J Clin Endocrinol Metab 2011;96:997–1005.
[9] Cascinu S, Verdecchia L, Valeri N, Berardi R, Scartozzi M. New target therapies in advanced pancreatic cancer. Ann Oncol 2006;17(Suppl. 5):v148–52.
[10] Scott AN, Hetheridge C, Reynolds AR, Nayak V, Hodivala-Dilke K, Mellor H. Farnesyltransferase inhibitors target multiple endothelial cell functions in angiogenesis. Angiogenesis 2008;11:337–46.
[11] Mijimolle N, Velasco J, Dubus P, Guerra C, Weinbaum CA, Casey PJ, et al. Protein farnesyltransferase in embryogenesis, adult homeostasis, and tumor development. Cancer Cell 2005;7:313–24.
[12] Potente M, Gerhardt H, Carmeliet P. Basic and therapeutic aspects of angiogenesis. Cell 2011;146:873–87.
[13] Gao J, Sun L, Huo L, Liu M, Li D, Zhou J. CYLD regulates angiogenesis by mediating vascular endothelial cell migration. Blood 2010;115:4130–7.
[14] Fan F, Schimming A, Jaeger D, Podar K. Targeting the tumor microenvironment: focus on angiogenesis. J Oncol 2011;2012:281261.
[15] Weis SM, Cheresh DA. Tumor angiogenesis: molecular pathways and therapeutic targets. Nat Med 2011;17:1359–70.
[16] Watanabe T, Noritake J, Kaibuchi K. Regulation of microtubules in cell migration. Trends Cell Biol 2005;15:76–83.
[17] Murphy DA, Courtneidge SA. The 'ins' and 'outs' of podosomes and invadopodia: characteristics, formation and function. Nat Rev Mol Cell Biol 2010;12:413–26.
[18] Li D, Xie S, Ren Y, Huo L, Gao J, Cui D, et al. Microtubule-associated deacetylase HDAC6 promotes angiogenesis by regulating cell migration in an EB1-dependent manner. Protein Cell 2011;2:150–60.
[19] Gao J, Huo L, Sun X, Liu M, Li D, Dong JT, et al. The tumor suppressor CYLD regulates microtubule dynamics and plays a role in cell migration. J Biol Chem 2008;283:8802–9.
[20] Zhou J, Vos CC, Gjyrezi A, Yoshida M, Khuri FR, Tamanoi F, et al. The protein farnesyltransferase regulates HDAC6 activity in a microtubule-dependent manner. J Biol Chem 2009;284:9648–55.
[21] Liu M, Aneja R, Sun X, Xie S, Wang H, Wu X, et al. Parkin regulates Eg5 expression by Hsp70 ubiquitination-dependent inactivation of c-Jun NH2-terminal kinase. J Biol Chem 2008;283:35783–88.
[22] Sun L, Gao J, Dong X, Liu M, Li D, Shi X, et al. EB1 promotes Aurora-B kinase activity through blocking its inactivation by protein phosphatase 2A. Proc Natl Acad Sci USA 2008;105:7153–8.
[23] Gerhardt H, Golding M, Fruttiger M, Ruhrberg C, Lundkvist A, Abramsson A, et al. VEGF guides angiogenic sprouting utilizing endothelial tip cell filopodia. J Cell Biol 2003;161:1163–77.
[24] Knabe W, Kuhn HJ. The role of microtubules and microtubule-organising centres during the migration of mitochondria. J Anat 1996;189(Pt 2):383–91.
[25] Shi X, Sun X, Liu M, Li D, Aneja R, Zhou J. CEP70 protein interacts with gamma-tubulin to localize at the centrosome and is critical for mitotic spindle assembly. J Biol Chem 2011;286:33401–08.
[26] Ballestrem C, Wehrle-Haller B, Hinz B, Imhof BA. Actin-dependent lamellipodia formation and microtubule-dependent tail retraction control-directed cell migration. Mol Biol Cell 2000;11:2999–3012.
[27] Suzuki N, Del Villar K, Tamanoi F. Farnesyltransferase inhibitors induce dramatic morphological changes of KNRK cells that are blocked by microtubule interfering agents. Proc Natl Acad Sci USA 1998;95:10499–504.
[28] Porzner M, Seufferlein T. Novel approaches to target pancreatic cancer. Curr Cancer Drug Targets 2011;11:698–713.
[29] Dong X, Liu F, Sun L, Liu M, Li D, Su D, et al. Oncogenic function of microtubule end-binding protein 1 in breast cancer. J Pathol 2010;220:361–9.
[30] Liu M, Wang X, Yang Y, Li D, Ren H, Zhu Q, et al. Ectopic expression of the microtubule-dependent motor protein Eg5 promotes pancreatic tumourigenesis. J Pathol 2010;221:221–8.
[31] Yarden Y, Baselga J, Miles D. Molecular approach to breast cancer treatment. Semin Oncol 2004;31:6–13.