出國報告（出國類別：其他 (兩岸會議)）

「第九屆海峽兩岸細胞生物學學術研討會」報告書

服務機關：國立中興大學生命科學系
姓名職稱：詹博兆 博士後研究員
派赴國家：中國
出國期間：2012 年 9 月 22 日至 9 月 29 日
報告日期：2012 年 12 月 21 日

摘要:

筆者受邀參加在中國雲南麗江召開第九屆海峽兩岸細胞生物學學術研討會 poster session，受邀的講者均為兩岸具有豐碩研究成果的第一線科研學者，因此這次的會議是一場高水平的學術研討會。首先在大會特別演講安排中國科學院上海巴斯德研究所孫兵所長開場，他的研究成果揭開 Th2 細胞利用 ECM-1 蛋白調控 sphingosine-1-phosphate receptor (S1P1)的表現，來促進 Th2 細胞遷移到發炎組織來產生免疫反應，因此 ECM-1 蛋白將來可以作為治療過敏以及氣喘的重要標的分子。接著台灣大學醫學院毒理學研究所郭明良教授說明 G9a 組蛋白甲基轉移酶在大腸癌幹細胞扮演重要的角色，並且暗示未來在治療大腸癌上 G9a 可以成為藥物開發的新標的。其它還有五個重要的細胞生物學主題分別進行四十餘場的口頭演講，還有第一次開放的壁報討論，與會學者經過熱烈的學術交流後，也建立起未來學術合作的橋樑。

目次:

目的:

海峽兩岸細胞生物學研討會是由中國科學院已故姚鑫院士與臺灣國家衛生研究院吳成文院長于1996年組織發起的雙邊學術會議,會議的目的是為了交流兩岸細胞生物學研究的最新成果，進一步促進海峽兩岸生物學科技與教育的合作與發展，採用每兩年在大陸和臺灣輪流召開的方式，迄今為止已舉行了8屆。每次的演講人由雙方各推舉20人左右組成，均為本領域內作出優秀成績的一線科研人員。因此，被遴選的演講人均把該機會視為業內同行對自己工作的肯定，已成為一種榮譽。

第8屆研討會期間，雙方認為應該增加交流頻率方能滿足兩岸細胞生物學發展的需要，因此決定自2012年起改為每年舉辦一次，仍由雙方輪流主辦。同時，為了拓展海峽會議的影響，讓更多的科技工作者參會，本次會議的每位報告人可邀請2位科技工作者列席會議。

本屆研討會由中國細胞生物學學會主辦，於 2012 年 9 月 23-26 日在雲南麗江舉行。筆者與中興大學生命科學院院長陳鴻震教授一同受邀參與本次研討會，除了報告最新的研究成果之外，會議期間也與中國傑出的科研學者建立起溝通的管道，未來不排除在學術上建立起合作的關係。

過程：

本屆海峽兩岸細胞生物學學術研討會 (The Ninth Across the Taiwan Strait Symposium on Cell Biology) 由中國細胞生物學學會 (Chinese Society for Cell Biology) 承辦，會議舉辦地點於中國雲南省麗江縣，時間為 2012 年 9 月 22 日至 29 日，筆者與中興大學生命科學院院長陳鴻震教授於 9 月 22 日由桃園機場經香港轉機抵達雲南省昆明市，再轉機至麗江市。9 月 23 日展開一連三天的學術會議，總計有 41 場的學術演講以及一場的壁報交流。 9 月 26 後的非正式學術交流主要是與中國學者討論未來在研究上建立實質合作機制的可能性之外，並且相互瞭解兩岸目前在細胞生物學研究上的現況以及建立雙方聯絡管道，共同尋求未來在科研計劃的實質合作，進一步商討雙方科研人員的互訪以及交換學者與學生的機制。我方人員最後於 9 月 28 日轉機至雲南昆明市， 隔日搭機經由香港回到臺灣。

這次的研討會主要包括了五大主題。首日的開幕式由中國同濟大學校長裴鋼院士和國家衛生研究院院長王陸海院士共同主持，之後大會安排的特別演講首先由中國科學院上海巴斯德研究所的孫兵聯合所長開場，介紹 ECM-1 蛋白是免疫細胞 Th2 移動的開關。臺灣大學郭明良教授與大家分享 G9a 組蛋白甲機轉移酶可以促進大腸癌細胞轉移，並且也是調節大腸癌幹細胞的重要分子。

第一個報告主題是**【幹細胞、細胞分化與發育生物學】**，總共有九位講者。陽明大學特聘教授吳成文院士向我們分享轉錄調節因子 CITED2 在生長因子 TGF-α 和 TGF-β 刺激之下，可以調節細胞生長以及靜默的功能。北京大學生命科學研究所高紹榮教授說明他們如何將分化的體細胞轉變為誘導多功能幹細胞 (iPS)，並且首次証明 iPS 細胞具有與胚胎幹細胞 (ES) 類似的真正多樣性。國家衛生研究院喻秋華研究員分享她如何在模式動物斑馬魚建立肝癌的研究模式。中國科學院裴端卿教授說明維生素 C 如何透過 judm1a/judm1b 酵素誘導體細胞轉化為幹細胞的分子機轉，並且解釋維生素 C 與組蛋白去甲基化酶的關聯。中研院孫以瀚研究員分享果蠅複眼與

觸角命運的分離機制。中科院李勁松教授是第一位成功建立孤雄單倍體胚胎幹細胞，並且可以在注入卵母細胞後產生健康的小鼠，這項研究成果將在哺乳動物遺傳學的研究，提供一個重要且操作方便的研究模式。台灣大學陳信孚教授介紹人類胚胎幹細胞、誘導多功能幹細胞的免疫原性，而復旦大學上海醫學院湯其群教授發現 BMP4 可以在白色脂肪組織誘導棕色脂肪的分子。游正博教授利用 glycoproteomics 以及 glycan 的方法，在乳癌幹細胞的細胞膜上找到全新未曾發現的糖蛋白，將來可以用來作為檢測乳癌的新標地。

當日下午進行兩個不同主題的演講以及一場壁報交流。**【醫學細胞生物學】**主題總計有八位講者，第一位由陽明大學生化所吳國瑞教授發現缺氧環境下，HDAC3 與 WDR5 的交互作用可以使上皮細胞轉變成間葉細胞 (EMT)。中國中山大學康鐵邦教授發表 GSK-3β 利用激活 NF-κB 路徑來促進骨肉瘤的形成，並且已針對 GSK-3β 進行腫瘤標把治療的臨床試驗。成功大學生理學研究所湯銘哲教授利用原子力顯微鏡測量正常細胞與癌細胞的軟硬度，發現大部份的腫瘤細胞比正常細胞來的軟，原因是癌細胞的細胞骨架蛋白已經發生異常。浙江大學醫學部李曉明教授在模式小鼠發現 NRG1/ErbB4 可以調節 PV 中間神經元的興奮性來影響癲癇的發生，並且 ErbB4 受體可能可以作為抗癲癇藥物的標地分子。國衛院裘正健研究員研究血液紊流對血管內皮細胞的分子變化，並指出 HDACs 的作用可能是造成動脈硬化的重要分子。山東大學神經生物學研究所陳哲宇教授發現 Rab11 是 BDNF 造成 TrkB 受體內吞作用的決定因子，進而再循環定位到突觸後區域。陽明大學生化所鄭子豪教授研究 polyQ 造成亨丁頓氏舞蹈症的病理機制，首次揭露 Spt4 是形成 polyQ 的關鍵分子。中國科學院劉新垣院士介紹癌症標靶基因病毒治療的策略以及超干擾素的抗癌作用以及原理。

第三個主題是**【細胞信號傳導】**，中國清華大學陳曄光教授發現 LIF 和 BMP 的協同作用可以調控 ERK 的活性，來保持胚胎幹細胞自我增生的能力。成功大學藥理

學研究所沈孟儒教授分享內質網蛋白 STIM1 如何與 Orai1 進行交互作用，進而促進子宮頸癌癌細胞的增生與移動。中科院陳雁教授說明一個位於高基氏體上的 RKTG 分子如何具有抑制腫瘤的功能。長庚大學張玉生教授發現 EB 病毒的 LMP1 蛋白可以透過與 PRA1 結合來活化 NF-κB，進而促進細胞移動。中科院宋保亮教授說明在肝臟中 gp78 蛋白透過 SREBP 的訊息傳遞路徑來調控脂質的合成。中興大學陳鴻震教授分享具有高度轉移能力的癌細胞如何利用 FAK 來組裝足體環 (podosome rosettes)。南京大學李朝軍教授揭露幼童遭受腮腺炎病毒感染後造成睪丸內 Sertoli 細胞內的蛋白進行不正常的 prenylation，可能是成年後不孕的原因。國衛院羅秀容教授利用酵母菌模式，找到第一個可以調節人類致病黴菌白色念珠菌藥物幫浦 CDR1 表現的轉錄因子 Ndt80。南京大學朱敏生教授透過建立基因剃除的模式小鼠，找到 MYPT1 分子可以影響腸道平滑肌的收縮。

第四個主題是**【免疫細胞生物學】**，首先由中科院錢友存教授說明介白素-17家族成員的功能與其機制。成功大學吳華林教授介紹單核球細胞膜上的 thrombomodulin，參與 LPS 和革蘭氏陰菌誘發的免疫發炎。武漢大學王延軼教授指出引發發炎反應的細胞激素可以透過 TRIM8 分子來調控 NF-κB 的活性。台灣大學分子醫學研究所徐立中教授利用 IKKβ 基因剃除小鼠，發現 PAI-2 具有調節內毒素誘發 IL-1β 形成的功能。中科院王盛典教授希望可以改善腫瘤免疫抑制的微環境，可以顯著提高 HER2/neu 抗體的腫瘤治療的效力。國衛院王澤華教授研究 MAP4Ks 家族成員在自體免疫疾病的角色，發現 GLK-PKC θ-IKK 可以作為 Th17 引起自體免疫疾病的診斷分子。中科院藍柯教授研究卡波氏肉瘤疱疹病毒的 miRNAs，發現 miR-K12-11 可以逃避宿主細胞的抗病毒天然免疫效應，穩定腫瘤疱疹病毒的潛伏感染。

第五個主題是**【植物細胞生物學】**，中國農業大學傅纓教授分享一個可與皮質微管結合的 REN2 分子如何調控植物細胞生長的方向。中興大學植病系葉錫東教授利用三種策略培育出可以抵抗病毒的木瓜品系。清華大學戚益軍教授發現 small RNAs

會參與去氧核糖核酸雙股斷裂的修復作用。中央研究院邱子珍教授揭曉 miR399 可以調控 PHO2 的表現，進而調節植物內磷酸鹽的平衡。中科院黃善金教授發現水稻 AIP1 蛋白可以與 ADF 和 profiling 的協同作用引起微絲的大量聚集，進而調控水稻的生長。台灣大學植物學研究所分享在阿拉伯芥不同組織中，銅鋅-SOD 活化的機轉。

筆者則受邀參加大會第一次舉辦的壁報學術交流，發表「P120RasGAP-Mediated Activation of c-Src Is Critical for Oncogenic Ras to Induce Tumor Invasion」研究成果，筆者在致癌性 Ras、p120RasGAP 以及 Src 等蛋白揭開過去從未報導的分子機轉，並且發現致癌性 Ras 所引起的腫瘤侵入及轉移需要倚賴 Src 的酵素活性上升來達成。Ras 在人類腫瘤中是最容易發生“成體細胞功能增加性突變”（somatic gain-of-function mutation）的標的基因。Ras 蛋白調控許多重要的訊息傳遞路徑，包括正常細胞的生長以及腫瘤細胞的惡性轉型（malignant transformation）。在本研究中，發現許多帶有致癌性突變 Ras（oncogenic Ras）的人類腫瘤細胞中，去除致癌性 Ras 的表現會同時抑制 Src 的酵素活性。相反地，在正常細胞表現致癌性 H-Ras、K-Ras 或者 N-Ras 都可以引起 Src 的酵素活性上升。並且致癌性 Ras 所引起 Src 的活性上升主要發生於高基氏體（Golgi complex），少部份位於內質網。透過慢病毒感染細胞表現 small hairpin RNAs（shRNAs）的篩選模式，我們找到致癌性 Ras 活化 Src 的關鍵分子 p120RasGAP （一種可以提高 Ras 的 GTPase 酵素活性的蛋白）。此外，發現致癌性 Ras 可以透過 p120RasGAP 來活化 Src，這是因為致癌性 Ras 可以吸引p120RasGAP 到高基氏體，並且進一步地促進p120RasGAP與Src的結合，因而導致 Src的酵素活性上升。最後發現致癌性 Ras 造成 Src 的活性上升可以選擇性地刺激並活化 JNK 訊息傳遞路徑，這對於致癌性 Ras 引起的細胞移動（cell motility）、細胞侵入（cell invasion）以及腫瘤轉移（metastasis）非常重要，但卻不太影響腫瘤生成（tumor growth）。發表會後，並與中國許多科研學者進行廣泛地討論，分享在學術研究上的心得與見解，包括中科院的劉新垣院士、中國山東理工大學生命科學系龐秋香教授、中國華東師範大學王平教授以及研究生 (如附件)，收穫良多。

心得與建議:

細胞生物學是生物學或醫學研究相當重要的基礎科學，藉由細胞生物學的研究，不但可以瞭解最根本生命現象的起源，還可以解釋人類許多疾病發生的原因，甚至在作物的改良與疾病的防治都可以提供重要的基礎，因此細胞生物學的研究一直是當今研究的重要課題。在這次的學術會議，除了瞭解中國學者在細胞生物學研究上有非常重要的研究成果之外，並且認識中國近年在基礎科學研究上的努力，不僅在人才招募、科研經費的投資上不餘遺力之外，並且訂定許多深具潛力以及獨具特色的研究方向，可以做為我們的借鏡。

除了兩場大會安排非常精彩的特別演講之外，在醫學細胞生物學主題所安排的內容，都是發表在國際一流期刊的學術報告。陽明大學生化暨分子生物研究所吳國瑞教授發現 HDAC3 透過 WDR5 增加 Histone H3 lysine 4 的甲基化，這是正常上皮細胞轉化為惡性間質細胞的關鍵。中國中山大學腫瘤防治中心康鐵邦研究員在腫瘤細胞發現 GSK-3B 失去作用後，因此無法促進 Cdc25A 降解，使得 Cdc25A 在腫瘤高表現的原因，目前康教授的研究團隊針對 GSK-3B 進行標把治療的臨床試驗。浙江大學醫學部李曉明教授發現 NRG1 結合到 ErbB4 受體後，可以活化 parvalbumin (PV) 中間神經元來影響癲癇的發生，並且透過動物實驗的結果暗示 ErbB4 受體可能是未來抗癲癇藥物的新標的。此外在植物細胞生物學的主題上，防治木瓜病毒的國際權威學者中興大學植物病理學系葉錫東教授，發表結合三種新穎基因轉殖策略來對植物病毒進行靜默作用 (silencing)，並且可以在田間達到病毒防治的效果，此一卓越研究成果令人印象深刻。

在正式會議後的非學術交流活動上，與會學者均廣泛地交換彼此的研究心得之外，也分享在研究工作上寶貴的經驗。此外，筆者認為透過面對面的學術交流活動容易建立起重要溝通的管道，對於日後的學術合作均有莫大的幫助。在未來除了可以繼續維持定期的學術會議，應可擴大在學術上的交流活動，其具體的作為如建立電子報，內容可以包括各項學術會議的召開、博士生以及博士後人才招募、訪問學者或交換學生的機制，甚至是學術研究的合作計畫，期望能夠共同提升華人在細胞生物學在國際上的學術地位。

附錄：

一、研討會議程

9 月 22 日（星期六）

16：00—17：30　註冊報到

地點：雲南麗江官房大酒店（雲南麗江古城香格里拉大道延伸段）

18：00—　自助晚餐

9 月 23 日（星期日）

09：00—09：30　參會代表註冊報到

09：30—10：00　開幕式/貴賓致詞　裴鋼/王陸海

10：00—12：00　大會特別演講　主持人：裴鋼、王陸海

10：00—11：00　Keynote Speaker 孫 兵（中國科學院上海巴斯德研究所）

ECM1 對 Th2 細胞遷移功能的影響

11：00—12：00　Keynote Speaker　郭明良（臺灣大學生命科學院/臺灣大學醫學院毒理學研究所）

The Role of Histone Methyltransferase G9a in Cancer and Colon Cancer Stem Cells

14：00 —17：20　Session I　幹細胞、細胞分化與發育生物學　主持人：伍焜玉、裴端卿

14：00—14：20　吳成文（“中研院”）

CITED2 functions as a molecular switch for cytokine-induced proliferation and quiescence

14：20—14：40　高紹榮（北京生命科學研究所）

體細胞重程式設計研究進展

14：40—15：00　喻秋華（衛生研究院分子與基因醫學研究所（臺灣））

From endoderm gene regulatory networks to liver cancer formation in zebrafish

15：00—15：20　裴端卿（中國科學院廣州生物醫藥與健康研究院）

Jhdm1a/1b 以維生素 C 依賴性提高重程式設計效率

15：20—15：40　Coffee Break

15：40—16：00　孫以瀚（“中研院”分子生物研究所）

Segregation of eye and antennal fates in Drosophila

16：00—16：20　李勁松（中科院上海生科院生化細胞所）

孤雄單倍體胚胎幹細胞注入卵母細胞後產生健康小鼠

16：20—16：40　陳信孚（台大醫院婦產部/臺灣大學醫學院臨床醫學研究所）

Immunogenicity of Human Pluripotent Stem Cells And The Derivatives

16：40—17：00　湯其群(復旦大學上海醫學院)

BMP4 on adipocyte development and metabolism

17：00—17：20　游正博（“中研院”細胞與個體生物學研究所）

Stem cells and cancer from the perspective of glycomics analysis

17：20—18：00　Poster Session 壁報交流

9 月 24 日（星期一）

09：00—12：00　Session Ⅱ　醫學細胞生物學　　主持人：張仲明、康鐵邦

09：00—09：20　吳國瑞(陽明大學生化暨分子生物研究所)
Interplay between HDAC3 and WDR5 is essential for hypoxia-induced epithelial-mesenchymal transition

09：20—09：40　康鐵邦(中山大學腫瘤防治中心)
細胞週期調控與腫瘤靶向治療

09：40—10：00　湯銘哲(成功大學醫學院生理學科暨生理所)
Mechanobiology of cancer cell transformation

10：00—10：20　李曉明(浙江大學醫學部)
受體酪氨酸激酶 ErbB4 在癲癇發病中的作用及其細胞分子機制

10：20—10：40　Coffee Break

10：40—11：00　裘正健(衛生研究院細胞及系統醫學研究所（臺灣）)
Vascular Endothelial Response to Disturbed Flow and Its Role in Atherogenesis

11：00—11：20　陳哲宇(山東大學醫學院)
神經元活性通過 Rab11 介導促進 TrkB 內吞後再迴圈定位到突觸後區域

11：20—11：40　鄭子豪(陽明大學生化暨分子生物研究所)
Spt4 Is Selectively Required for Transcription of Extended Trinucleotide Repeats

11：40—12：00　劉新垣（中科院上海生科院生化細胞所）
癌症治療新策略及其機理

14：00 —17：20　Session Ⅲ 細胞信號轉導　　主持人：陳 雁、賴明詔

14：00—14：20　陳曄光(清華大學生命科學學院)
Modulation of mouse embryonic stem cell fate by BMP

14：20—14：40　沈孟儒(成功大學醫學院藥理所暨婦產學科)
Calcium signaling, cell tension and cancer progression

14：40—15：00　陳　雁(中國科學院上海生命科學研究院營養所)
From spatial regulation of cell signaling to physiological homeostasis

15：00—15：20　張玉生(長庚大學生物醫學研究所)
Epstein-Barr virus-encoded latent membrane protein 1 interacts with PRA1 to activate NF-kB signaling and intercellular trafficking for promoting cell migration

15：20—15：40　Coffee Break

15：40—16：00　宋保亮(中國科學院上海生命科學研究院生物化學與細胞生物學研究所)
小腸膽固醇吸收的分子機制研究

16：00—16：20　陳鴻震(中興大學生命科學系所)
Role of FAK in assembly of podosome rosettes

16：20—16：40　李朝軍(南京大學醫學院)
Mumps-related adult infertility is associated with a protein prenylation alteration in Sertoli cells caused by viral infection during childhood

16：40—17：00　羅秀容(衛生研究院感染症與疫苗研究所（臺灣）)

Ndt80p a Transcriptional Regulator Involved in Stress Response in Human Fungal Pathogen Candida albicans

17：00—17：20　朱敏生(南京大學模式動物研究所)

Alteration of Contractile Phenotypes of Intestinal Smooth Muscle in Myosin Phosphatase Target Subunit 1 Deficient Mice

9 月 25 日（星期二）

09：00—11：40　Session Ⅳ　免疫細胞生物學　　主持人：孫 兵、謝世良

09：00—09：20　錢友存(中國科學院上海生命科學研究院健康科學研究所)

白介素-17 家族細胞因子的功能與機制研究

09：20—09：40　吳華林(成功大學醫學院生物化學暨分子生物研究所)

Monocytic thrombomodulin triggers LPS- and Gram-negative bacteria-induced inflammatory response

09：40—10：00　王延軼(中國科學院武漢病毒研究所)

TRIM8 modulates NF-κB activation pathways triggered by proinflammatory cytokines

10：00—10：20　徐立中(臺灣大學醫學院分子醫學研究所)

The role of PAI-2 in endotoxin-induced IL-1b production

10：20—10：40　Coffee Break

10：40—11：00　王盛典(中國科學院生物物理研究所)

誘導腫瘤相關巨噬細胞極化增強抗 HER2/neu 抗體腫瘤治療效應

11：00—11：20　譚澤華(衛生研究院免疫醫學研究中心（臺灣）)

Roles of MAP4Ks in Lymphocyte Signaling and Human Autoimmune Diseases

11：20—11：40　藍 柯(中國科學院上海巴斯德研究所)

腫瘤皰疹病毒 KSHV 編碼 miRNA 的功能研究

14：00 —16：20　Session Ⅴ植物細胞生物學　　主持人：戚益軍、吳華林

14：00—14：20　傅 纓(中國農業大學)

A novel Arabidopsis microtubule associated protein, REN2, plays an important role in regulation of anisotropic cell growth

14：20—14：40　葉錫東(中興大學植物病理學系)

CD147-interacting molecules and their signal pathways

14：40—15：00　戚益軍(清華大學生命科學學院)

A role for small RNAs in DNA double-strand break repair

15：00—15：20　Coffee Break

15：20—15：40　邱子珍(“中研院”農業生物科技研究中心)

Shear Stress Regulation of Vascular Endothelial Cell-Smooth Muscle Cell Interactions

15：40—16：00　黃善金(中國科學院植物研究所)

AIP1 通過促進微絲動態周轉調控水稻生長發育

16：00—16：20　靳宗洛(臺灣大學植物科學研究所)

CuZnSOD Activation Pathway in Arabidopsis

16：20—17：20　全體參會代表合影留念

9 月 26 日—9 月 27 日（星期三至星期四）　學術交流

二、筆者詹博兆博士發表之專題論文

P120RasGAP-Mediated Activation of c-Src Is Critical for Oncogenic Ras to Induce Tumor Invasion

Abstract

Ras genes are the most common targets for somatic gain-of-function mutations in human cancers. In this study, we found a high incidence of correlation between Ras oncogenic mutations and c-Src activation in human cancer cells. We demonstrated that oncogenic Ras induces c-Src activation mainly on the Golgi complex and endoplasmic reticulum. Moreover, we identified p120RasGAP as an effector for oncogenic Ras to activate c-Src. The recruitment of p120RasGAP to the Golgi complex by oncogenic Ras facilitated its interaction with c-Src, thereby leading to c-Src activation, and this p120RasGAP-mediated activation of c-Src was important for tumor invasion induced by oncogenic Ras. Collectively, our findings unveil a relationship between oncogenic Ras, p120RasGAP, and c-Src, suggesting a critical role for c-Src in cancers evoked by oncogenic mutations in *ras* genes.

Key words: Ras, Src, p120RasGAP, invasion

Introduction

The human Ras proteins – H-Ras, N-Ras, K-Ras (K-Ras4A, K-Ras4B) – are the most well-known members of the Ras family. They have been extensively studied for over 30 years partly because of their high frequency of activating mutations in human cancers (1). Ras proteins function as GDP/GTP molecular switches that regulate diverse signaling pathways and cell functions (1). Cycling between GDP- and GTP-bound states of Ras is controlled by two classes of regulatory molecules: guanine nucleotide exchange factors (GEFs) and GTPase-activating proteins (GAPs) (2). GEFs facilitate the intrinsic GDP/GTP exchange activity of Ras, whereas GAPs stimulate the relatively slow intrinsic GTP hydrolysis activity of Ras. GTP-bound Ras regulates a complex signaling network by binding to and activating diverse classes of effector molecules, such as Raf, phosphatidylinositol 3-kinase (PI3K), and RalGEFs (3).

Ras emanates signals not only from the plasma membrane but also from organelles, including the Golgi complex, the endoplasmic reticulum (ER), mitochondria, and endosomes (4). The biological significance for compartmentalized Ras signaling was

initially described in T lymphocytes, in which the activation of Ras-MAPK signaling at different subcellular compartments could lead to either negative or positive selection of T lymphocytes (5). The compartmentalized Ras signaling is also conserved in fission yeasts. Ras1p, a single Ras protein in *Schizosaccharomyces pombe*, regulates mating from the plasma membrane and cell morphology from the endomembranes (6). Therefore, the biological functions elicited by Ras are closely related to its subcellular localizations.

Cellular Src (c-Src), a non-receptor protein tyrosine kinase, has been implicated in the regulation of a variety of cellular functions (7, 8). To exert such diverse biological activities, c-Src interacts with and phosphorylates a wide range of cellular proteins (7). Crystallographic analysis has revealed that the Src-homology (SH) 2 and SH3 domains of c-Src bind to its own tyrosine 527 and a short polyproline type II helix between the SH2 and kinase domain, leaving c-Src in a close, autoinhibited state (9). Interactions of c-Src with other cellular proteins via its SH2 and/or SH3 domains could disrupt the intramolecular inhibitory interactions, resulting in c-Src autophosphorylation on tyrosine 416 in the activation loop within the kinase domain for its full activation (7). Although c-Src is frequently found to be hyper-activated in human cancers, its genetic mutations are rarely observed (10).

It has been known for years that oncogenic Src (v-Src) can lead to Ras activation through different modes. For example, v-Src can phosphorylate the adaptor protein Shc, which then recruits Grb2/Sos complexes for Ras activation (11). Additionally, Ras has been demonstrated to be essential for v-Src-stimulated cell transformation (12). However, it is unclear whether oncogenic Ras could lead to the activation of c-Src. In particular, as activation of Ras and c-Src are frequently found in human cancers, clarification of their causal relationship will be of importance for clinical implication. In this study, we found that oncogenic Ras induces c-Src activation predominantly on the Golgi complex and ER. In addition, p120RasGAP was identified as an effector for oncogenic Ras to induce c-Src activation. Importantly we demonstrated that the p120RasGAP–mediated activation of c-Src is critical for oncogenic Ras to promote tumor invasion.

Results

Oncogenic Ras induces c-Src activation

The possibility for oncogenic Ras to activate c-Src was first examined in human cancer cell lines that harbor oncogenic mutations in *ras* genes (14). Depletion of oncogenic Ras (K-Ras, H-Ras, or N-Ras) by short-hairpin RNA (shRNA) decreased c-Src phosphoY416 (Src pY416) in 71% (10/14) of examined cell lines (Fig. 1A; Table 1). The decreased Src pY416 caused by H-Ras knockdown was rescued by re-expression of

oncogenic H-Ras^{L61} (Fig. 1B), indicating that the decreased Src pY416 is not because of a nonspecific off-target effect of shRNA. Moreover, H-Ras^{N17}, which is known to effectively inhibit all three Ras isoforms by sequestration of Ras GEFs (15), was capable of suppressing Src pY416 in T24 cells (with oncogenic H-Ras), SW480 cells (with oncogenic K-Ras), and HT-1080 cells (with oncogenic N-Ras) (Fig. 1C). Alternatively, an overexpression strategy was undertaken to examine if the forced expression of oncogenic Ras could lead to c-Src activation. We found that transient, stable, or induced expression of oncogenic Ras stimulated the catalytic activity of c-Src, accompanied by increased Src pY416 (Figs. 1D, E, and F). Together, our results clearly indicate that oncogenic Ras leads to c-Src activation. It is worth noting that c-Src activation by Ras might be specific to oncogenic forms of Ras, since depletion of endogenous Ras did not affect c-Src activation by platelet-derived growth factor (Fig. 1G) or cell adhesion (Fig. 1H).

Oncogenic Ras activates c-Src mainly on the Golgi complex and ER

Ras is known to localize to different membrane compartments via post-translational modifications, such as farnesylation or palmitoylation, on its carboxyl terminus (4). A selective inhibitor (FTI-277) for farnesyltransferase was found to inhibit the activation of c-Src by H-Ras^{V12} (Supplementary Fig. S1A), suggesting that the association of H-Ras^{V12} with cellular membranes may be important for its ability to activate c-Src. To test this possibility, c-Src activation by H-Ras^{L61} and its variants deficient in association with different membrane compartments was evaluated. The subcellular localization of H-Ras^{L61} and its variants was visualized by green fluorescent protein (GFP)-fused Raf Ras-binding domain (Raf-RBD) (Supplementary Fig. S1B). H-Ras$^{L61/C186S}$ (defective in farnesylation), which fails to associate with any cellular membranes, lost its ability to activate Src (Fig. 2A). H-Ras$^{L61/C181S/C184S}$ (defective in palmitoylation), which is known to associate with the Golgi complex and ER, but not the plasma membrane (16), retained its ability to activate Src to an extent similar to that activated by H-Ras^{L61} (Fig. 2A). To further confirm that the association of H-Ras^{L61} with the Golgi complex and/or ER is important for it to activate c-Src, H-Ras$^{L61/C186S}$ was engineered to target to the Golgi complex or ER. The Golgi-targeted H-Ras$^{L61/C186S}$ (KDELR-Ras$^{L61/C186S}$) and the ER-targeted H-Ras$^{L61/C186S}$ (M1-Ras$^{L61/C186S}$) substantially activated c-Src to the level comparable to that by Ras^{L61} (Fig. 2A), indicating that the association of H-Ras^{L61} with the Golgi complex and ER is important for it to activate c-Src. Similarly, we found that K-Ras$^{V12/C185S}$, which cannot be farnesylated, failed to activate c-Src (Fig. 2B). By contrast, K-Ras$^{V12/S181E}$, which is known to predominantly associate with endomembranes (17), activated c-Src more potently than K-Ras^{V12} (Fig. 2B). Therefore, our results suggest that association of oncogenic Ras with endomembranes, in particular the Golgi complex and ER, may be crucial for it to activate c-Src.

To detect the subcellular localization where c-Src is activated by oncogenic Ras,

MDCK or Cos cells transiently expressing HA epitope-tagged H-Ras^{V12} (HA-H-Ras^{V12}) were stained for active Src with anti-Src pY416. Our results showed that active, endogenous c-Src was detected only in the cells expressing HA-H-Ras^{V12} (Fig. 2C), in which it was co-localized with HA-H-Ras^{V12} at the Golgi complex (Fig. 2D) and ER (Fig. 2E). Notably, although a fraction of HA-H-Ras^{V12} was distributed at the plasma membrane, no active Src was detected at the plasma membrane (Fig. 2C, arrow heads). Our results thus suggest that the Golgi complex and ER may be the major platforms for oncogenic Ras to activate c-Src.

It is known that myristoylation of Src at Gly2 is required for its association with cellular membranes (18). We employed c-Src-GFP (with a GSGS-linker between c-Src and GFP) and its G2A mutant to examine whether membrane association is required for c-Src to be activated by oncogenic Ras. We found that c-Src-GFP was retained in an inactive state and mainly resided at the perinuclear region in resting cells (Supplementary Fig. S1C). Upon stimulation by epidermal growth factor, c-Src-GFP became activated and was recruited to the plasma membrane (Supplementary Fig. S1C), indicating that the activity and subcellular localization of c-Src-GFP can be regulated in response to extracellular stimuli. Unlike c-Src-GFP, c-Src^{G2A}-GFP was diffusely distributed in the cytoplasm and was refractory to be activated by H-Ras^{V12} (Supplementary Fig. S1D). Together, our results suggest that membrane tethering of oncogenic Ras and c-Src on the Golgi complex and ER may be essential for oncogenic Ras to activate c-Src.

Activation of c-Src by oncogenic Ras is not through alterations in ROS production, autocrine, or cell adhesion

How does oncogenic Ras induce c-Src activation? First, we examined whether reactive oxygen species (ROS) play a role in this regard. Oncogenic Ras was reported to induce large amount of ROS (19). Because ROS has been shown to stimulate the activity of Src (8, 20), we speculated that oncogenic Ras might activate c-Src via ROS production. However, this possibility was excluded, because elimination of ROS by N-acetyl-cysteine (NAC), a potent ROS scavenger, did not prevent the activation of c-Src by H-Ras^{V12} (Supplementary Figs. S2A and B). Second, we speculated an autocrine mechanism might be involved in Src activation by oncogenic Ras. However, the conditioned medium collected from the cells expressing oncogenic Ras did not stimulate c-Src activity, rendering it less likely that c-Src activation by oncogenic Ras is through an autocrine fashion (Supplementary Figs. S2C and D). Third, we examined whether oncogenic Ras activates c-Src through its effect on cell adhesion. We found that oncogenic Ras was able to activate c-Src even when the cells were kept in suspension (Supplementary Figs. S2E and F), thus indicating that activation of c-Src by oncogenic Ras cannot be attributed to cell adhesion.

Oncogenic Ras activates c-Src independently of PI3K, Raf, RalGEF, or Ral

PI3K, Raf, and RalGEF are the three most well-known immediate effectors for Ras (21). We found that inhibition of PI3K by specific inhibitors (wortmannin and LY294002) or depletion of the p110 catalytic subunit of PI3K by shRNA did not impair the ability of oncogenic Ras to activate c-Src (Supplementary Figs. S3A and B). In addition, the inhibition of Raf and its downstream effector MEK by the inhibitors ZM336372 and PD98059 did not prevent c-Src activation by oncogenic Ras (Supplementary Fig. S3C). Moreover, H-Ras$^{V12/S35}$ and H-Ras$^{V12/G37}$, which preferentially activate Raf and RalGEF, respectively, (22), activated c-Src to a level similar to that by H-Ras^{V12} (Supplementary Fig. S3D), thus suggesting that activation of Raf and RalGEF by oncogenic Ras does not lead to c-Src activation. The role of Ral in c-Src activation was then further examined. Overexpression of constitutively active RalA or RalB had little effect on c-Src activity (Supplementary Fig. S3E). RLIP76△GAP is a dominant-negative construct for both RalA and RalB (23). We found that neither expression of RLIP76△GAP nor depletion of RalA and RalB affected the activation of c-Src by oncogenic Ras (Supplementary Figs. S3F, G and H). Therefore, our results indicate that PI3K, Raf, RalGEF, and Ral are less likely to be involved in Ras-induced activation of c-Src.

P120RasGAP is a key mediator for oncogenic Ras to activate c-Src

To identify the molecule(s) essential for oncogenic Ras to activate c-Src, a collection of shRNAs that target Ras effectors, GEFs and GAPs, was applied to our study. Through an unbiased screening, we identified p120RasGAP (RasGAP) as an effector for oncogenic Ras to activate c-Src. We found that knockdown of p120RasGAP reduced Src pY416 in 79% (11/14) of examined cancer cell lines (Fig. 3A; Table 1). Knockdown of both K-Ras and p120RasGAP had more profound inhibition in c-Src activation than knockdown of either one in SW480 cells, (Fig. 3B), supporting that oncogenic Ras, RasGAP, and c-Src are in the same signaling axis. The reduced c-Src activity by p120RasGAP knockdown was rescued by expression of GFP-fused p120RasGAP in H-Ras^{V12}-transformed NIH3T3 cells (Fig. 3C and D). Moreover, knockdown of p120RasGAP impaired the ability of H-Ras^{L61} to activate c-Src (Fig. 3E). Conversely, overexpression of p120RasGAP, but not its R789Q mutant deficient in Ras binding (24), potentiated the ability of H-Ras^{L61} to activate c-Src (Fig. 3F), indicating that binding of p120RasGAP to oncogenic Ras is important for c-Src activation. Of note, the Q938H mutant lacking GAP activity (24) was able to potentiate the ability of H-Ras^{L61} to activate c-Src (Fig. 3F), indicating that the GAP activity of p120RasGAP is not required for it to promote the activation of c-Src by oncogenic Ras.

Oncogenic Ras interacts with c-Src via p120RasGAP

H-Ras^{L61}, but not H-Ras$^{L61/C186S}$, forms stable complexes with endogenous c-Src and

p120RasGAP in intact cells (Fig. 4A). The interaction between H-Ras^{L61} and c-Src was specific because c-Src was not co-precipitated with constitutively active Rho^{V14} or Rac^{V12} (Supplementary Fig. S4A). As GTP-bound H-Ras does not directly bind to c-Src *in vitro* (Supplementary Fig. S4B), it is possible that p120RasGAP may mediate the interaction between oncogenic Ras and c-Src. Indeed, depletion of p120RasGAP by shRNAs markedly reduced the association of c-Src with Ras^{L61} (Fig. 4B) and the Golgi-targeted Ras$^{L61/C186S}$ (Fig. 4C). Overexpression of p120RasGAP, but not its R789Q mutant defective in Ras binding, promoted the interaction between Ras^{L61} and c-Src (Fig. 4D).

Moreover, we found that H-Ras^{L61} promoted the interaction between p120RasGAP and c-Src (Fig. 4E). In HT-1080 cancer cells, suppression of oncogenic Ras by Ras^{N17} or specific shRNA inhibited the binding of p120RasGAP to c-Src (Figs. 4F and G). Thus, our results support a role for oncogenic Ras to facilitate the interaction between p120RasGAP and c-Src. In accordance with this notion, we found that in the absence of oncogenic Ras, endogenous p120RasGAP was distributed throughout the cytoplasm in a tubule-like pattering and did not co-localize with the Golgi marker KDELR-GFP (Fig. 4H). By contrast, in the presence of H-Ras^{V12}, p120RasGAP became condensed and co-localized with KDELR-GFP at the perinuclear region (Fig. 4H), where it was co-localized with active c-Src (Fig. 4I). *In vitro*, p120RasGAP bound to both SH2 and SH3 domains of c-Src (Supplementary Fig. S4C). Deletion of the amino-terminal proline-rich region in p120RasGAP significantly reduced its interaction with the SH3 domain of Src (Supplementary Fig. S4D). Together, our results support a model that recruitment of p120RasGAP to the Golgi complex by oncogenic Ras facilitates the interaction of p120RasGAP with c-Src, which may alleviate the intramolecular inhibitory conformation of c-Src, leading to c-Src activation.

P120RasGAP-medaited activation of c-Src is essential for oncogenic Ras to promote tumor invasion

To examine the functional significance of c-Src activation in the transforming potential of oncogenic Ras, H-Ras^{V12} was co-expressed with or without c-Src in SYF (src$^{-/-}$ yes$^{-/-}$ fyn$^{-/-}$) cells (Fig. 5A). We found that H-Ras^{V12} by itself did not promote proliferation of SYF cells (Fig. 5B), but it was sufficient to support anchorage-independent growth of SYF cells in soft agar (Fig. 5D) and allowed them to form tumors in mice (Fig. 5E). However, H-Ras^{V12} by itself failed to stimulate invasive and metastatic capabilities of SYF cells (Figs. 5F, G, and H). H-Ras^{V12} was capable of conferring invasive and metastatic potential to the cells only in the presence of c-Src (Figs. 5F, G, and H), strongly supporting a critical role for c-Src in tumor invasion induced by oncogenic Ras.

To examine the significance of p120RasGAP in oncogenic Ras-induced malignant transformation, p120RasGAP was depleted in human SW480 colon cancer cells that express oncogenic K-Ras. Depletion of p120RasGAP by shRNA significantly decreased

c-Src activity (Fig. 6A) and impaired the invasiveness of the cells (Fig. 6E), but had no effect on cell proliferation, survival, and anchorage-independent growth (Figs. 6B, C, and D). Expression of GFP-p120RasGAP restored the c-Src activity and invasiveness in p120RasGAP-depleted SW480 cells (Figs. 6A and E). These results suggest that c-Src activation may be essential for oncogenic Ras to promote cell invasion, but not anchorage-independent cell growth. It is worth noting that knockdown of p120RasGAP did not decrease the invasiveness of LoVo cells (Fig. S5), in which c-Src activity is independent of p120RasGAP (Fig. S5A; Table 1). These results together highlight the significance of the p120RasGAP-Src axis in tumor invasion evoked by oncogenic Ras.

Discussion

Because Ras and c-Src represent two major molecular switches for intracellular signal transduction, understanding their interplay will help us not only to realize the intracellular signaling network linked by these two molecular switches, but also to delineate more effective strategies for future therapeutic intervention. In this study, we uncover a new signaling pathway that links oncogenic Ras to c-Src activation. Through a shRNA-based screening, we identified p120RasGAP as a key mediator for oncogenic Ras to activate c-Src. As summarized in Fig. 6F, p120RasGAP acts as a negative regulator for normal Ras by stimulating GTP hydrolysis. However, in cancer cells, oncogenic Ras facilitates the interaction of p120RasGAP with c-Src on the Golgi complex, which may induce conformational changes in c-Src, leading to its activation. Our results suggest that p120RasGAP-mediated activation of c-Src may be essential for oncogenic Ras to induce tumor invasion, but not anchorage-independent cell growth.

Although p120RasGAP serves as a negative regulator for normal Ras, it has been implicated as an effector for oncogenic Ras (25-27). For instance, p120RasGAP was demonstrated to be important for oncogenc Ras to induce cell transformation (25). In particular, interruption of the interaction between oncogenic Ras and p120RasGAP was shown to suppress Ras-induced transformation (26). However, the direct targets for p120RasGAP in Ras-induced transformation were unclear. In this study, we demonstrated that p120RasGAP functions as an effector for oncogenic Ras to activate c-Src. Our results indicated that the Ras-binding capability of p120RasGAP, but not its GAP activity, is required for oncogenic Ras to activate c-Src (Fig. 3F). In addition, we showed that p120RasGAP is recruited to the Golgi complex upon the expression of oncogenic Ras (Fig. 4H). We thus propose that recruitment of p120RasGAP to the Golgi complex by binding to oncogenic Ras may cause conformational changes in p120RasGAP, thereby exposing its binding sites to c-Src. Moreover, we found that the expression level of p120RasGAP is up-regulated by oncogenic Ras (Supplementary Fig. S6), which could further contribute to

Ras-induced cell transformation. P120RasGAP has been shown to interact with p190RhoGAP (28). In this study, we found that depletion of p190RhoGAP does not affect c-Src activation by oncogenic Ras (Fig. S7), suggest that p190RhoGAP is not involved in c-Src activation in context with oncogenic Ras.

In this study, we showed that c-Src activation is crucial for oncogenic Ras to stimulate tumor invasion. This leads to the question of how active c-Src promotes tumor invasion in context with oncogenic Ras. It is possible that active c-Src on the Golgi complex may enhance the secretion of matrix metalloproteinases through the regulation of exocytosis. It was shown recently that active c-Src phosphorylates and activates dynamin 2 to induce marked Golgi fragmentation and vesicle transport from the Golgi to the plasma membrane during secretory processes (29). In addition, Golgi-associated c-Src may promote cell migration and invasion through its effect on protein glycosylation (30). As alterations in the glycans of membrane proteins could lead to changes in cell adhesion and migration (31, 32), it is possible that the Golgi-associated c-Src may alter protein glycosylation in a way that is beneficial for tumor invasion.

We demonstrated in this study that expression of oncogenic Ras induces c-Src activation. In addition, we found that there is a high correlation between oncogenic Ras and c-Src activation in human cancer cell lines that harbor oncogenic mutations in *ras* genes (Table 1). In accordance with our findings, Shields et al. (33) recently reported that elevated Src activity is detected in more than 60% of patients with pancreatic ductal adenocarcinoma, which is characterized with a high incidence of oncogenic mutations in the *K-ras* gene. In this study, we found that knockdown of oncogenic Ras or p120RasGAP by shRNAs led to suppression in c-Src activity in 79% of examined cell lines harboring oncogenic Ras mutations. Given that Ras is the most common target for somatic gain-of-function mutations in human cancers, clarification of the role of c-Src in Ras-dependent malignancy is important for determining clinical implication and may be helpful for the development of therapeutic strategies.

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Table 1. Effect of knockdown of Ras or p120RasGAP on Src activity in human cancer cell lines

Cell lines	Origin	Ras mutation[1]	Src inhibition by Ras knockdown	Src inhibition by RasGAP knockdown
T24	Bladder carcinoma	H-Ras G12V	yes	yes
HT-1080	Fibrosarcoma	N-Ras Q61K	yes	yes
SW480	Colorectal adenocarcinoma	K-Ras G12V	yes	yes
NCI-H460	Lung carcinoma	K-Ras Q61H	yes	yes
HOP-62	Lung carcinoma	K-Ras G12C	yes	yes
NCI-H23	Lung adenocarcinoma	K-Ras G12S	yes	yes
MDA-MB-231	Breast adenocarcinoma	K-Ras G13D	yes	yes
MIA PaCa-2	Pancreatic carcinoma	K-Ras G12C	yes	yes
PANC-1	Pancreatic carcinoma	K-Ras G12D	yes	no
AsPC-1	Pancreatic carcinoma	K-Ras G12D	yes	no
HCT-116	Colorectal carcinoma	K-Ras G13D	no[2]	yes
A549	Lung adenocarcinoma	K-Ras G12S	no	yes
NCI-H358	Lung carcinoma	K-Ras G13C	no	yes
LoVo	Colorectal adenocarcinoma	K-Ras G13D	no	no

[1]Ras mutation as described in Davies *et al*, 2002 (14).

[2]Src activity is suppressed by H-Ras^{N17}.

Figure 1. Oncogenic Ras induces c-Src activation.

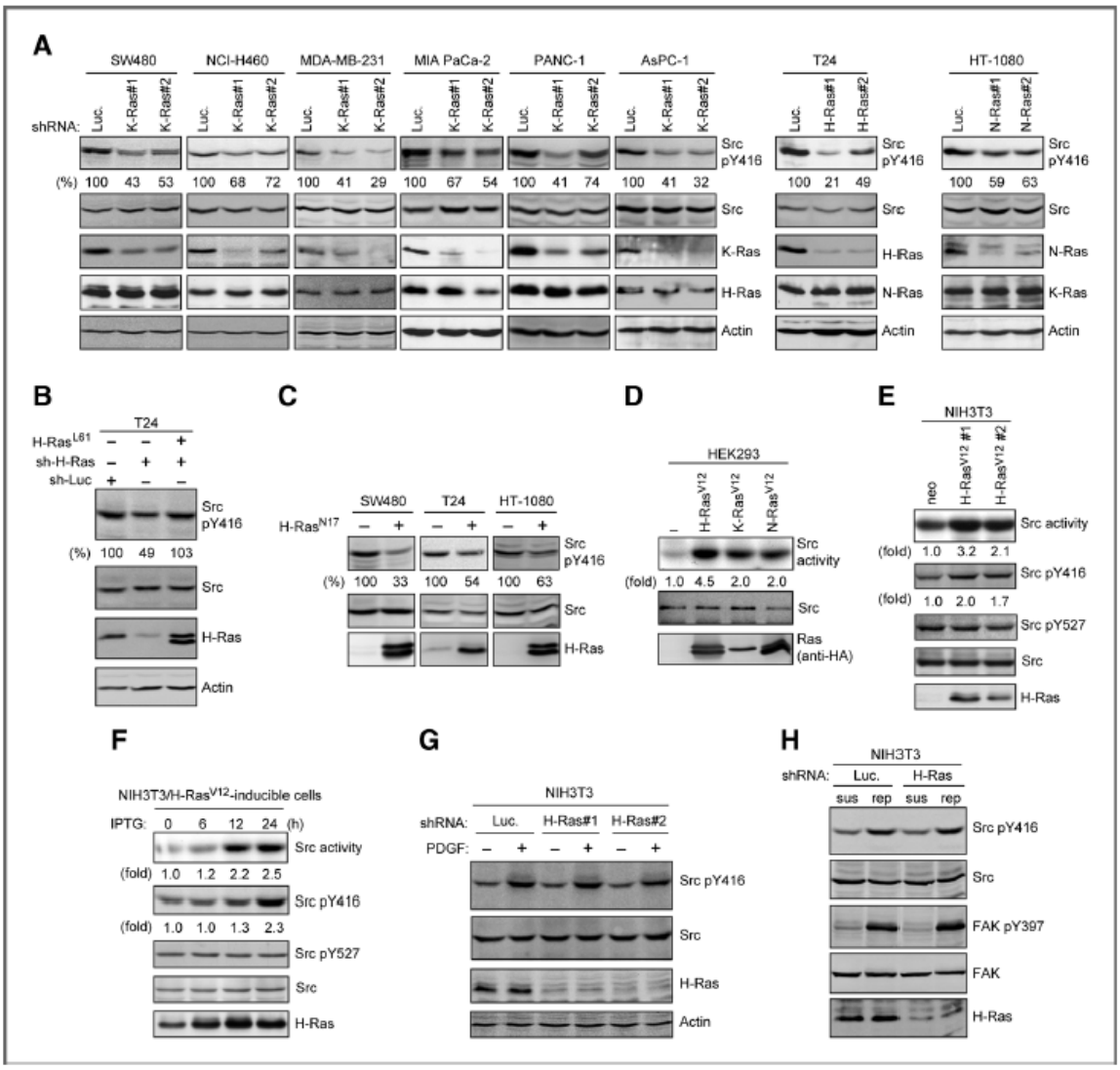


Figure 1. Oncogenic Ras induces c-Src activation. A, human cancer cell lines harboring oncogenic Ras were infected with recombinant lentiviruses encoding shRNAs specific to oncogenic Ras or luciferase (Luc) as a control. Two shRNAs (#1 and #2) were used to knockdown oncogenic Ras. The level of Src pY416 was analyzed by immunoblotting with anti-Src pY416. B, inhibition of Src pY416 by sh-H-Ras in T24 cells was rescued by reexpression of H-Ras^{L61}. C, H-Ras^{N17} was transiently expressed in cancer cells and its effect on Src pY416 was measured. D, HA-tagged Ras^{V12} constructs were transiently expressed in HEK293 cells and their effect on c-Src activity was analyzed by an *in vitro* kinase assay with acid-denatured enolase as a substrate. The expression of HA-tagged Ras was analyzed by immunoblotting with anti-HA. E, cell lysates from 2 NIH3T3 cell clones (#1 and #2) stably expressing H-Ras^{V12} and a neomycin-resistant control clone (neo) were subjected to analysis for the activity and pY416 of c-Src. F, an isopropyl-β-D-1-thiogalactopyranoside (IPTG)-inducible expression system for H-Ras^{V12} was established in NIH3T3 cells. H-Ras^{V12} was allowed to express for various times and its effect on the activity of pY416 and pY527 of c-Src was analyzed. G, endogenous H-Ras of NIH3T3 cells was depleted by shRNA and its effect on platelet-derived growth factor (PDGF)–induced activation of c-Src was measured. H, endogenous H-Ras of NIH3T3 cells was depleted by shRNA and its effect on cell adhesion–induced activation of c-Src was measured. The cells were kept in suspension (sus) and then replated (rep) on fibronectin-coated dishes. Focal adhesion kinase (FAK) pY397 was used as an indicator for cellular responses to cell adhesion.

Figure 2. Oncogenic Ras induces c-Src activation mainly on the Golgi complex and ER.

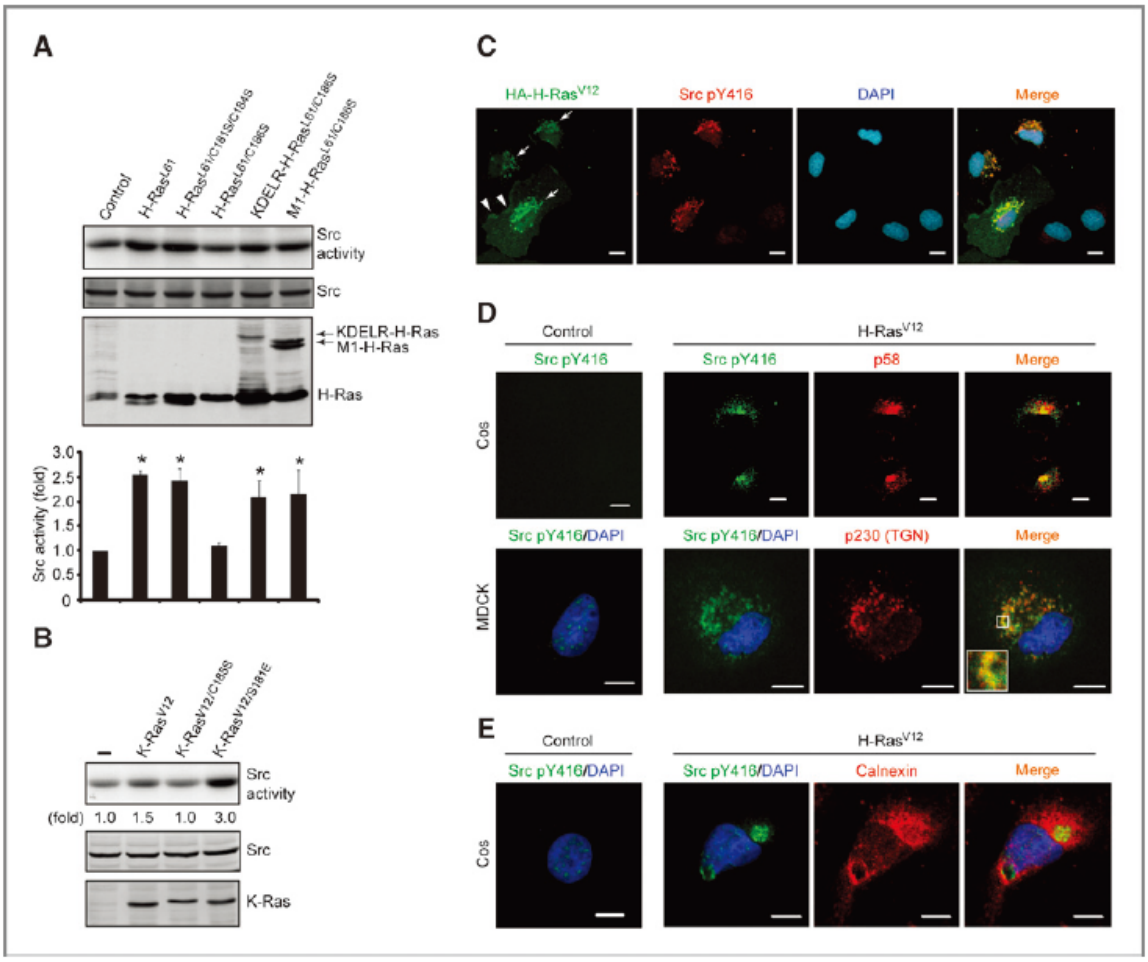


Figure 2. Oncogenic Ras induces c-Src activation mainly on the Golgi complex and ER. A, H-Ras^{L61} and its variants were transiently expressed in HEK293 cells and their effect on c-Src activity was analyzed. *, $P < 0.01$. B, K-Ras^{V12} and its variants were transiently expressed in HEK293 cells and their effect on c-Src activity was analyzed. C, HA-H-Ras^{V12} was transiently expressed in MDCK cells. The cells were subjected to immunofluorescent staining for HA-H-Ras and Src pY416. Note that the endogenous, active c-Src detected by anti-Src pY416 is colocalized with endomembrane-associated H-Ras^{V12} (arrows), but not plasma membrane–associated H-Ras^{V12} (arrowheads). Scale bar, 10 μm. D, H-Ras^{V12} was transiently expressed in Cos cells or MDCK cells. The cells were stained for the Golgi complex and Src pY416, with p58 as a marker for *cis*-Golgi and p230 as a marker for trans-Golgi. The selected area (white box) in MDCK cells was enlarged. Scale bar, 10 μm. E, H-Ras^{V12} was transiently expressed in Cos cells. The cells were stained for the ER and Src pY416, with calnexin as a marker for the ER. Scale bar, 10 μm. DAPI, 4′,6-diamidino-2-phenylindole.

Figure 3. p120RasGAP is a key mediator for oncogenic Ras to activate c-Src.

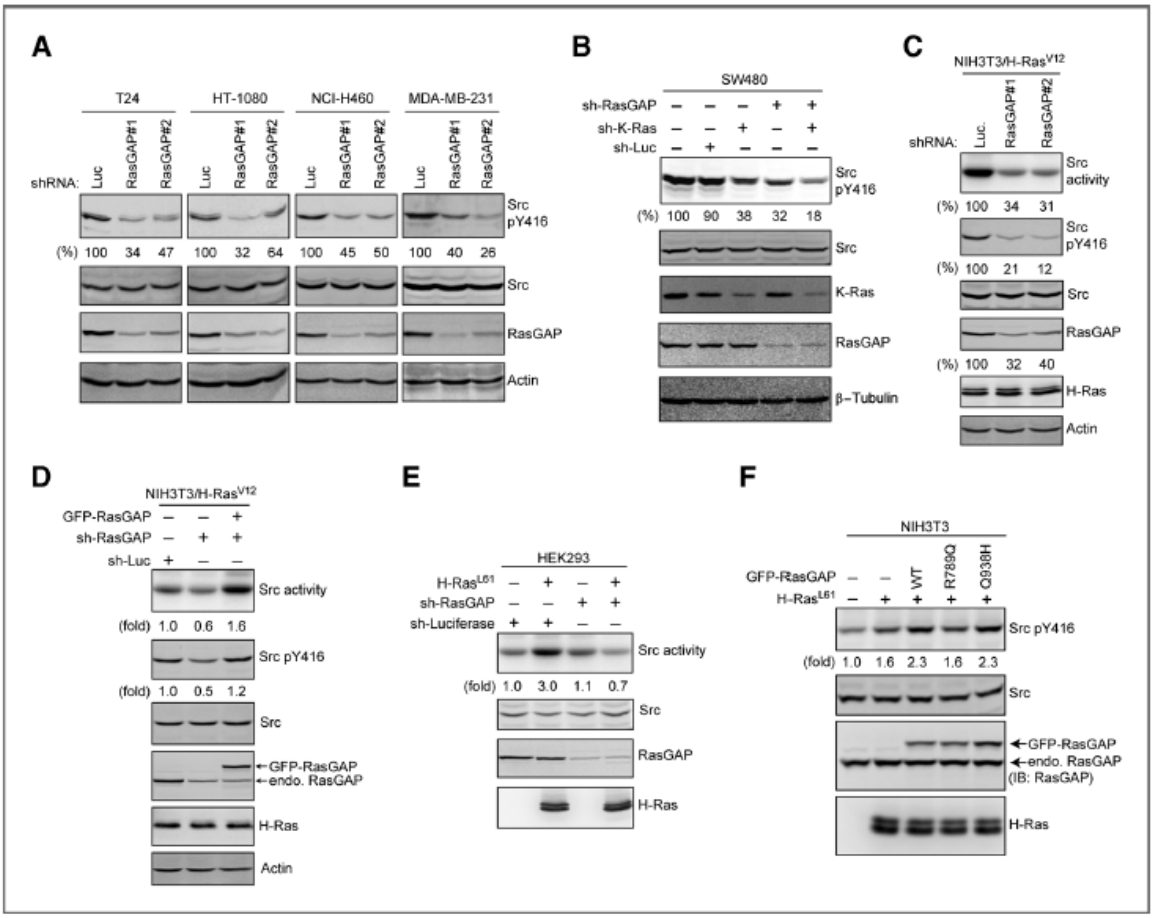


Figure 3. p120RasGAP is a key mediator for oncogenic Ras to activate c-Src. A, p120RasGAP was depleted by shRNAs in human cancer cell lines and its effect on Src pY416 was analyzed. Two shRNAs (#1 and #2) specific to p120RasGAP were used. An shRNA to luciferase (Luc) was used as a control. B, K-Ras and/or p120RasGAP were depleted by shRNAs in SW480 cells and the effect on Src pY416 was analyzed. C, p120RasGAP was depleted in H-Ras^{V12}–transformed NIH3T3 cells and its effect on the pY416 and activity of Src was measured. D, inhibition of Src pY416 and activity by p120RasGAP shRNA (sh-RasGAP) was rescued by expression of GFP-p120RasGAP (GFP-RasGAP). E, H-Ras^{L61} failed to stimulate c-Src activity in the cells expressing p120RasGAP shRNA. F, GFP-p120RasGAP or its mutants were transiently expressed in NIH3T3 cells and their effect on Src pY416 was measured. The R789Q mutant is defective in Ras binding. The Q938H mutant lacks the GAP activity. IB, immunoblotting; WT, wild-type

Figure 4. Oncogenic Ras interacts with c-Src via p120RasGAP.

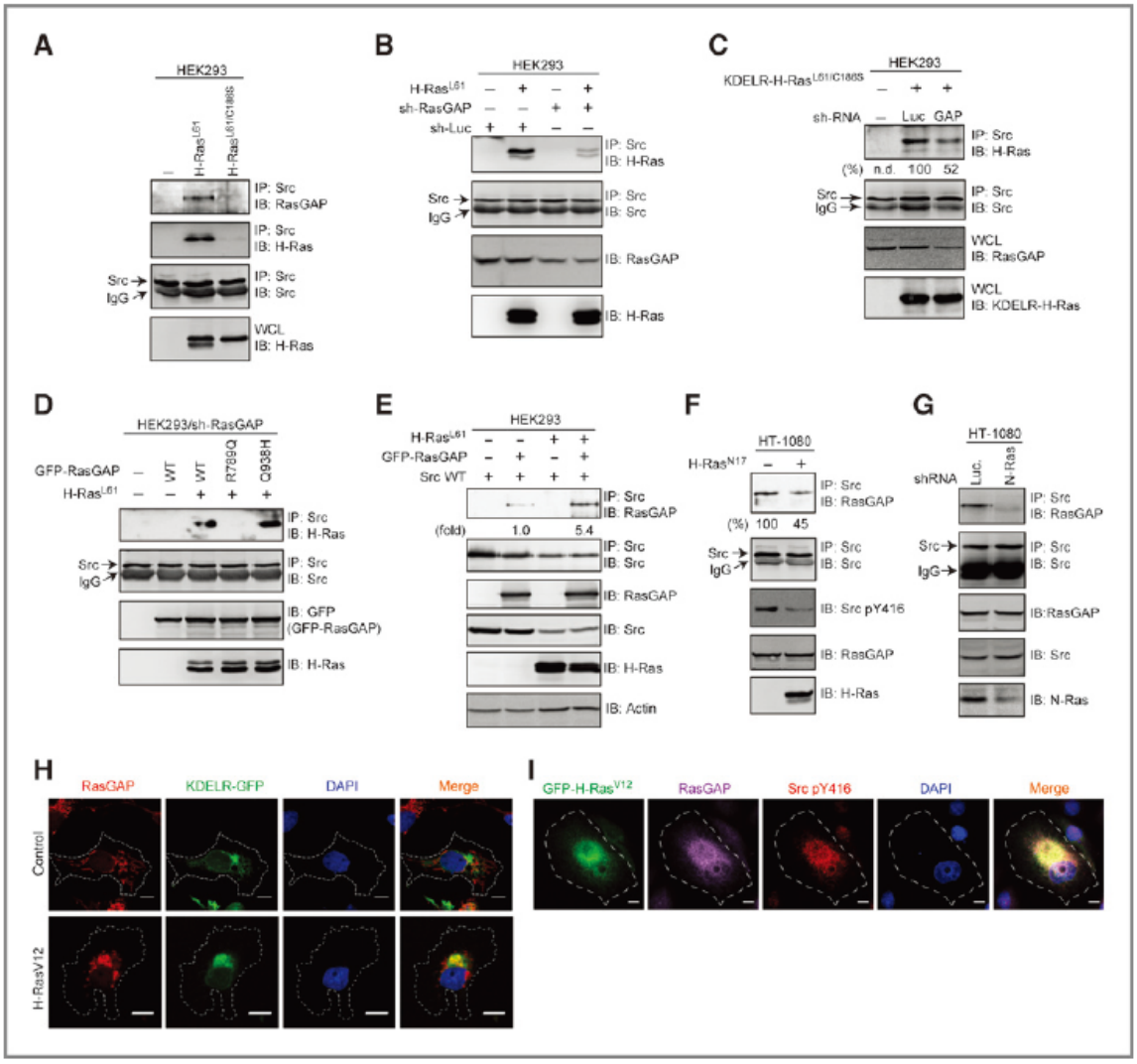


Figure 4. Oncogenic Ras interacts with c-Src via p120RasGAP. A, oncogenic Ras is associated with endogenous c-Src in intact cells. Ras^{L61} or $Ras^{L61/C186S}$ was transiently expressed in HEK293 cells and the lysates were subjected to immunoprecipitation (IP) and immunoblotting (IB) with antibodies, as indicated. WCL, whole-cell lysates. B, knockdown of p120RasGAP by shRNA (sh-RasGAP) suppresses the association of H-Ras^{L61} with endogenous c-Src. H-Ras^{L61} was transiently expressed in HEK293 cells stably expressing shRNAs to p120RasGAP or luciferase (Luc). The association of H-Ras^{L61} with Src was analyzed. C, the association of the Golgi-targeted Ras mutant (KDELR-H-$Ras^{L61/C186S}$) with Src was partially suppressed by p120RasGAP knockdown. D, H-Ras^{L61} and GFP-p120RasGAP were transiently expressed in HEK293 cells stably expressing shRNA to p120RasGAP. The association of H-Ras^{L61} with Src was analyzed. E, H-Ras^{L61} enhances the interaction between p120RasGAP and c-Src. HEK293 cells were transiently transfected with plasmids, as indicated, and the association of Src with p120RasGAP was analyzed. F, H-Ras^{N17} was transiently expressed in HT-1080 cells and its effect on the interaction between p120RasGAP and c-Src was analyzed. G, N-Ras was depleted in HT-1080 cells and the interaction between p120RasGAP and c-Src was analyzed. H, Cos cells and those stably expressing H-Ras^{V12} were transiently transfected with the plasmid encoding KDELR-GFP. The cells were stained for p120RasGAP and the nucleus. KDELR-GFP was used as an indicator for the Golgi complex. Note that whereas p120RasGAP is scatter distributed in the control cells, it is condensed at the Golgi complex in the cells expressing H-Ras^{V12}. The dashed lines mark the outline of the cell. Scale bar, 10 μm. I, GFP-H-Ras^{V12} was transiently expressed in Cos cells. The cells were stained for p120RasGAP and Src pY416. Note that GFP-H-Ras^{V12} colocalizes with p120RasGAP and active Src at the perinuclear region. The dashed lines mark the outline of the cell. Scale bar, 10 μm. DAPI, 4′,6-diamidino-2-phenylindole; n.d., not determined.

Figure 5. c-Src is essential for H-Ras^{V12} to stimulate cell invasion and metastasis.

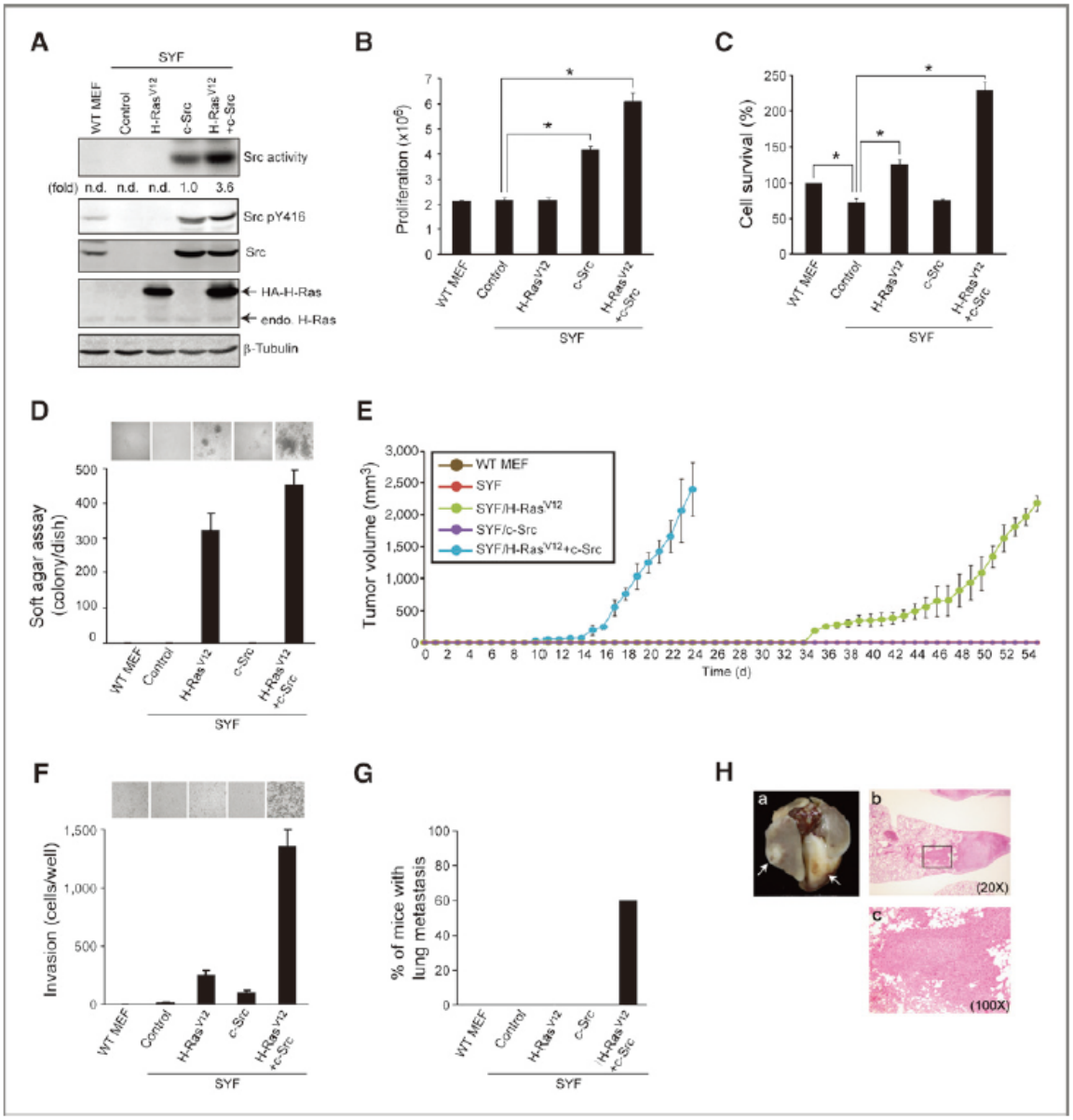


Figure 5. c-Src is essential for H-Ras^{V12} to stimulate cell invasion and metastasis. A, lysates from wild-type MEFs (WT MEF) and SYF cells (src$^{-/-}$ yes$^{-/-}$ fyn$^{-/-}$) stably expressing HA-tagged H-Ras^{V12} (SYF/H-Ras^{V12}), c-Src (SYF/c-Src), or both (SYF/c-Src + H-Ras^{V12}) were subjected to analysis for the activity and pY416 of Src. B–E, the stable cell lines as described in A were subjected to assays for cell proliferation (B), survival (C), soft agar colony formation assay (D), and tumorigenicity in nude mice (E). Results are presented as mean ± SD. ($n = 3$). *, $P < 0.01$. F and G, the stable cell lines as described in A were subjected to assays for cell invasion (F) and lung metastasis (G). Results are presented as mean ± SD. ($n = 3$). H, lungs were excised from the mice injected with the SYF cells expressing both H-Ras^{V12} and c-Src and then fixed in formalin. Representative images for the lung (a) and hematoxylin and eosin (H&E) staining (b and c) are shown. Arrows indicate tumor nodules. n.d., not determined.

Figure 6. p120RasGAP is essential for oncogenic Ras to promote tumor invasion.

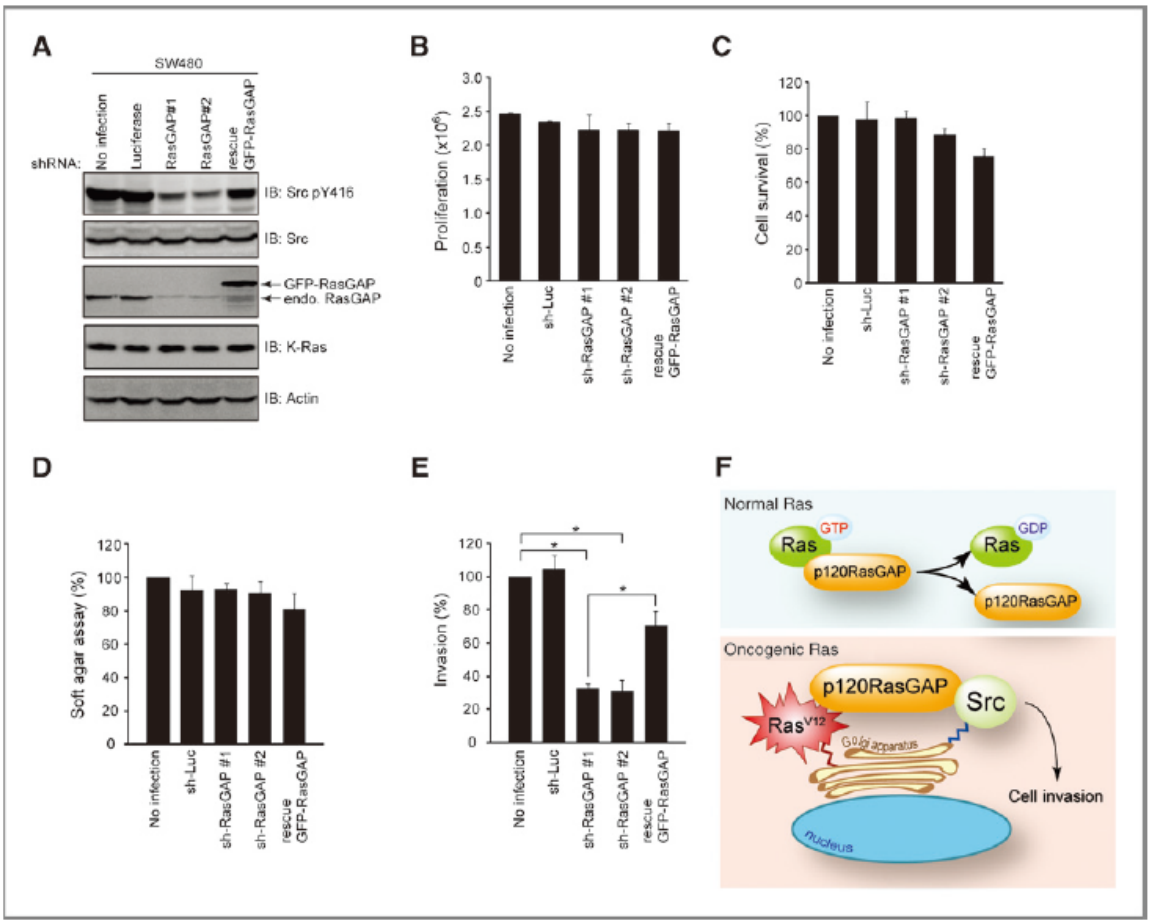
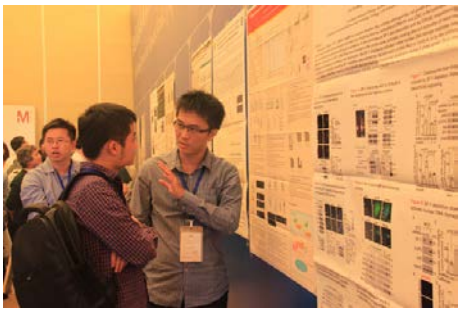


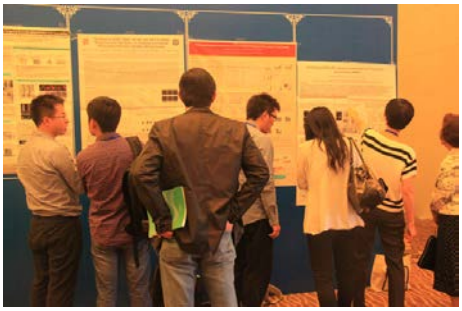
Figure 6. p120RasGAP is essential for oncogenic Ras to promote tumor invasion. A, p120RasGAP was depleted by shRNAs (#1 and #2) in SW480 cells. GFP-p120RasGAP was introduced into the cells expressing shRNA #1 (rescue GFP-RasGAP). The whole-cell lysates were analyzed by immunoblotting (IB) with antibodies, as indicated. B–E, SW480 cells as described in A were subjected to assays for cell proliferation (B), survival (C), soft agar colony formation assay (D), and cell invasion (E). Results are presented as mean ± SD. ($n = 3$).*, $P < 0.01$. F, a model illustrating that oncogenic Ras activates c-Src through p120RasGAP on the Golgi complex (bottom), whereas normal Ras is negatively regulated by GTP hydrolysis through p120RasGAP (top).

三、活動照片

1. 筆者與中國中山大學腫瘤防治中心學者進行壁報交流



2. 筆者與中國山東理工大學生命科學系龐秋香副教授進行壁報交流



3. 口頭演講現場

4. 兩岸與會學者合照