

Desmocollin-2 (DSC2) Inhibit proliferation and migration ability in human lung cancer cells

黃士庭、蔡孟

E-mail: 354902@mail.dyu.edu.tw

ABSTRACT

Lung cancer is the most common cause of cancer death in the world, and lung cancer patients death mainly due to metastasis. The process of metastasis have many complex mechanisms involved, consist of many different functional genes. The main purpose of this study is screening with lung cancer metastasis-related genes, and thus further explore the molecular mechanisms of cancer metastasis. Previously, using microarray assay and lung cancer metastasis model cell lines, screening with cancer metastasis-related genes. Additionally, we focus on cancer metastasis-related gene, Desmocollin-2. In this study, Desmocollin-2 expression in highly metastatic lung cancer cell lines (such as A549 and CL1-5) is fewer than low metastatic lung cancer cell (CL1-0), these result have proved that Desmocollin-2 gene expression levels in lung cancer cells were negatively correlation with the metastasis activity of a panel of lung cancer cell lines. Using shRNA-DSC2 transfection methods, we suggested that knockdown DSC2 gene expression can promote lung cancer cells proliferation and migration, at the same time inhibit Desmocollin-2 gene expression also promote lung cancer cells colonyforming. Finally found that stable suppression Desmocollin-2 gene in cells whose morphology similar Epithelial to mesenchymal transition (EMT) phenomenon, These result have proved that Desmocollin-2 may play a role in tumor suppressor.

Keywords : lung cancer、metastasis、Desmocollin-2、shRNA、Epithelial to mesenchymal transition

Table of Contents

封面內頁 簽名頁 中文摘要.....	
..... iii 英文摘要.....	
..... iv 誌謝	
..... v 目錄	
..... ix 圖目錄.....	
..... xii 1. 前言.....	
..... 1 1.1 癌症.....	
..... 1 1.2 肺癌.....	
..... 1 1.3 致癌基因與腫瘤抑制基因.....	
..... 1 1.4 癌轉移與相關基因的研究.....	
..... 6 1.5 Desmosome.....	
..... 9 1.6 Desmocollin-2 (DSC2) 基因和已知的功能.....	
..... 11 1.7 Desmocollin-2 (DSC2) 基因和癌症之間的研究.....	
..... 12 1.8 -catenin.....	
..... 14 1.9 EGFR.....	
..... 15 2. 研究動機.....	
..... 18 3. 實驗設計與流程.....	
..... 19 4. 材料方法.....	
..... 20 4.1 細胞株.....	
..... 20 4.2 細胞繼代.....	
..... 20 4.3 細胞凍存.....	21
4.4 轉染.....	
.... 21 4.5 RNA萃取.....	
.... 22 4.6 RNA去除DNA處理.....	
.... 22 4.7 cDNA合成反應.....	
.... 23 4.8 即時定量PCR.....	
.... 23 4.9 建立抑制DSC2表現之系統.....	
.... 24 4.10 菌種保存.....	

.....	25 4.11 質體DNA萃取.....
..... 25 4.11.1 傳統法質體DNA萃取.....
..... 25 4.11.2 質體DNA之kit萃取.....
..... 26 4.12 DNA電泳.....
..... 27 4.13 DNA膠體kit純化.....
..... 28 4.14 MTT assay.....
..... 28 4.15 Colony formation assay.....
..... 29 4.16 細胞遷移分析 (Cell migration assay).....
..... 29 4.16.1 wound healing assay.....
..... 29 4.16.2 Transwell migration assay
..... 30 4.17 西方墨點分析 (Western Blot)
..... 31 4.16.1 SDS膠體的製備.....
..... 31 4.16.2 蛋白質樣品製備.....
..... 32 4.16.3 蛋白質的定量.....
..... 32 4.16.4 SDS膠體電泳.....
..... 33 4.16.5 電轉.....
..... 33 4.16.6 抗體雜合.....
..... 34 4. 結果.....
..... 36 4.1 透過microarray assay的分析比較CL1-5以及CL1-0細胞中的基因表現情形.....
..... 36 4.2 藉由即時定量PCR的方式分析CL1-5以及CL1-0肺癌細胞中的基因表現情形.....
..... 36 4.3 以即時定量PCR的方式分析Desmocollin-2-ab在侵入能力不同的細胞株中mRNA表現量的差異.....
..... 36 4.4 針對Desmocollin-2的兩種異構物以即時定量PCR的方式分析不同的細胞株中表現量的差異.....
..... 37 4.5 以西方墨點法分析Desmocollin-2在侵入能力不同的肺癌細胞株中蛋白質表現量的差異.....
..... 37 4.6 以即時定量PCR的方式分析shDSC2的抑制效果.....
..... 38 4.7 以wound healing assay 來分析其Transient transfection shDSC2-497、pGIPZ的CL1-0細胞的移動能力.....
..... 39 4.8 以colonyformation assay 分析Transient transfection shDSC2、pGIPZ的CL1-0細胞群落形成的能力.....
..... 39 4.9 以MTT assay分析Transient transfection shDSC2、pGIPZ的CL1-0細胞的增生速度.....
..... 40 4.10 以即時定量PCR的方式檢測轉染shDSC2的CL1-0細胞株.....
..... 40 4.11 以西方墨點法分析穩定抑制Desmocollin-2基因mRNA的細胞株.....
..... 41 4.12 以transwell migration分析穩定抑制Desmocollin-2基因的CL1-0細胞株.....
..... 41 4.13 以colonyformation assay分析穩定抑制Desmocollin-2基因的CL1-0細胞株其細胞群落形成的能力.....
..... 42 4.14 以MTT assay分析穩定抑制Desmocollin-2基因的CL1-0細胞株增生的速度.....
..... 42 4.15 分析穩定抑制Desmocollin-2基因的CL1-0細胞株增生情形.....
..... 43 4.16 觀察穩定抑制Desmocollin-2基因的CL1-0細胞型態.....
..... 43 5. 結論.....
..... 45 參考文獻.....
..... 66 附錄.....

REFERENCES

- 1.Al Moustafa AE, Alaoui-Jamali MA, Batist G, Hernandez-Perez M, Serruya C, Alpert L, Black MJ, Sladek R, Foulkes WD: Identification of genes associated with head and neck carcinogenesis by cDNA microarray comparison between matched primary normal epithelial and squamous carcinoma cells. *Oncogene* 2002, 21(17):2634-2640.
- 2.Alberg AJ, Samet JM: Epidemiology of lung cancer. *Chest* 2003, 123(1 Suppl):21S-49S.
- 3.Anami K, Oue N, Noguchi T, Sakamoto N, Sentani K, Hayashi T, Hinoh T, Okajima M, Graff JM, Yasui W: Search for transmembrane protein in gastric cancer by the Escherichia coli ampicillin secretion trap: expression of DSC2 in gastric cancer with intestinal phenotype. *J Pathol* 2010, 221(3):275-284.
- 4.Anand P, Kunnumakkara AB, Sundaram C, Harikumar KB, Tharakan ST, Lai OS, Sung B, Aggarwal BB: Cancer is a preventable disease that requires major lifestyle changes. *Pharm Res* 2008, 25(9):2097-2116.
- 5.Batlle E, Sancho E, Franci C, Dominguez D, Monfar M, Baulida J, Garcia De Herreros A: The transcription factor snail is a repressor of E-cadherin gene expression in epithelial tumour cells. *Nat Cell Biol* 2000, 2(2):84-89.
- 6.Bell DA: Origins and molecular pathology of ovarian cancer. *Mod Pathol* 2005, 18 Suppl 2:S19-32.
- 7.Bell DW, Varley JM, Szydlo TE, Kang DH, Wahrer DC, Shannon KE, Lubratovich M, Verselis SJ, Isselbacher KJ, Fraumeni JF et al: Heterozygous germ line hCHK2 mutations in Li-Fraumeni syndrome. *Science* 1999, 286(5449):2528-2531.
- 8.Bellacosa A, Chan TO, Ahmed NN, Datta K, Malstrom

S, Stokoe D, McCormick F, Feng J, Tsichlis P: Akt activation by growth factors is a multiple-step process: the role of the PH domain. *Oncogene* 1998, 17(3):313-325. 9.Beug H: Breast cancer stem cells: eradication by differentiation therapy? *Cell* 2009, 138(4):623-625. 10.Bhattacharjee RN, Banks GC, Trotter KW, Lee HL, Archer TK: Histone H1 phosphorylation by Cdk2 selectively modulates mouse mammary tumor virus transcription through chromatin remodeling. *Mol Cell Biol* 2001, 21(16):5417-5425. 11.Biesalski HK, Bueno de Mesquita B, Chesson A, Chytil F, Grimble R, Hermus RJ, Kohrle J, Lotan R, Norpeth K, Pastorino U et al: European Consensus Statement on Lung Cancer: risk factors and prevention. *Lung Cancer Panel. CA Cancer J Clin* 1998, 48(3):167-176; discussion 164-166. 12.Brabletz T, Jung A, Reu S, Porzner M, Hlubek F, Kunz-Schughart LA, Knuechel R, Kirchner T: Variable beta-catenin expression in colorectal cancers indicates tumor progression driven by the tumor environment. *Proc Natl Acad Sci U S A* 2001, 98(18):10356-10361. 13.Brabletz T, Jung A, Spaderna S, Hlubek F, Kirchner T: Opinion: migrating cancer stem cells - an integrated concept of malignant tumour progression. *Nat Rev Cancer* 2005, 5(9):744-749. 14.Bronner CE, Baker SM, Morrison PT, Warren G, Smith LG, Lescoe MK, Kane M, Earabino C, Lipford J, Lindblom A et al: Mutation in the DNA mismatch repair gene homologue hMLH1 is associated with hereditary non-polyposis colon cancer. *Nature* 1994, 368(6468):258-261. 15.Carpenter G: Receptors for epidermal growth factor and other polypeptide mitogens. *Annu Rev Biochem* 1987, 56:881-914. 16.Carpenter G, Cohen S: 125I-labeled human epidermal growth factor. Binding, internalization, and degradation in human fibroblasts. *J Cell Biol* 1976, 71(1):159-171. 17.Carpenter G, Cohen S: Epidermal growth factor. *Annu Rev Biochem* 1979, 48:193-216. 18.Carvalho A, Carmena M, Sambade C, Earnshaw WC, Wheatley SP: Survivin is required for stable checkpoint activation in taxol-treated HeLa cells. *J Cell Sci* 2003, 116(Pt 14):2987-2998. 19.Catelinois O, Rogel A, Laurier D, Billon S, Hemon D, Verger P, Tirmarche M: Lung cancer attributable to indoor radon exposure in france: impact of the risk models and uncertainty analysis. *Environ Health Perspect* 2006, 114(9):1361-1366. 20.Cavallaro U, Christofori G: Cell adhesion and signalling by cadherins and Ig-CAMs in cancer. *Nat Rev Cancer* 2004, 4(2):118-132. 21.Chen J, Den Z, Koch PJ: Loss of desmocollin 3 in mice leads to epidermal blistering. *J Cell Sci* 2008, 121(Pt 17):2844-2849. 22.Chen L, Chan TH, Yuan YF, Hu L, Huang J, Ma S, Wang J, Dong SS, Tang KH, Xie D et al: CHD1L promotes hepatocellular carcinoma progression and metastasis in mice and is associated with these processes in human patients. *J Clin Invest* 2010, 120(4):1178-1191. 23.Chen X, Cheung ST, So S, Fan ST, Barry C, Higgins J, Lai KM, Ji J, Dudoit S, Ng IO et al: Gene expression patterns in human liver cancers. *Mol Biol Cell* 2002, 13(6):1929-1939. 24.Cheng X, Koch PJ: In vivo function of desmosomes. *J Dermatol* 2004, 31(3):171-187. 25.Chidgey M: Desmosomes and disease: an update. *Histol Histopathol* 2002, 17(4):1179-1192. 26.Chidgey M, Brakebusch C, Gustafsson E, Cruchley A, Hail C, Kirk S, Merritt A, North A, Tselepis C, Hewitt J et al: Mice lacking desmocollin 1 show epidermal fragility accompanied by barrier defects and abnormal differentiation. *J Cell Biol* 2001, 155(5):821-832. 27.Christensen AH, Benn M, Bundgaard H, Tybjaerg-Hansen A, Haunso S, Svendsen JH: Wide spectrum of desmosomal mutations in Danish patients with arrhythmogenic right ventricular cardiomyopathy. *J Med Genet* 2010, 47(11):736-744. 28.Christiansen JJ, Rajasekaran AK: Reassessing epithelial to mesenchymal transition as a prerequisite for carcinoma invasion and metastasis. *Cancer Res* 2006, 66(17):8319-8326. 29.Christofori G: New signals from the invasive front. *Nature* 2006, 441(7092):444-450. 30.Chu YW, Yang PC, Yang SC, Shyu YC, Hendrix MJ, Wu R, Wu CW: Selection of invasive and metastatic subpopulations from a human lung adenocarcinoma cell line. *Am J Respir Cell Mol Biol* 1997, 17(3):353-360. 31.Clevers H: Wnt/beta-catenin signaling in development and disease. *Cell* 2006, 127(3):469-480. 32.Collins JE, Legan PK, Kenny TP, MacGarvie J, Holton JL, Garrod DR: Cloning and sequence analysis of desmosomal glycoproteins 2 and 3 (desmocollins): cadherin-like desmosomal adhesion molecules with heterogeneous cytoplasmic domains. *J Cell Biol* 1991, 113(2):381-391. 33.Collins JE, Taylor I, Garrod DR: A study of desmosomes in colorectal carcinoma. *Br J Cancer* 1990, 62(5):796-805. 34.Collins LG, Haines C, Perkel R, Enck RE: Lung cancer: diagnosis and management. *Am Fam Physician* 2007, 75(1):56-63. 35.Comijn J, Berx G, Vermassen P, Verschueren K, van Grunsven L, Bruyneel E, Mareel M, Huylebroeck D, van Roy F: The two-handed E box binding zinc finger protein SIP1 downregulates E-cadherin and induces invasion. *Mol Cell* 2001, 7(6):1267-1278. 36.Cristofanilli M, Braun S: Circulating tumor cells revisited. *JAMA* 2010, 303(11):1092-1093. 37.Culkins CC, Setzer SV: Spotting Desmosomes: The First 100 Years. *J Invest Dermatol* 2007, 127(E1):E2-E3. 38.Darnell JE, Jr., Kerr IM, Stark GR: Jak-STAT pathways and transcriptional activation in response to IFNs and other extracellular signaling proteins. *Science* 1994, 264(5164):1415-1421. 39.De Bortoli M, Beffagna G, Baucé B, Lorenzon A, Smaniotto G, Rigato I, Calore M, Li Mura IE, Basso C, Thiene G et al: The p.A897KfsX4 frameshift variation in desmocollin-2 is not a causative mutation in arrhythmogenic right ventricular cardiomyopathy. *Eur J Hum Genet* 2010, 18(7):776-782. 40.Dhanasekaran SM, Barrette TR, Ghosh D, Shah R, Varambally S, Kurachi K, Pienta KJ, Rubin MA, Chinnaiyan AM: Delineation of prognostic biomarkers in prostate cancer. *Nature* 2001, 412(6849):822-826. 41.Donis-Keller H, Dou S, Chi D, Carlson KM, Toshima K, Lairmore TC, Howe JR, Moley JF, Goodfellow P, Wells SA, Jr.: Mutations in the RET proto-oncogene are associated with MEN 2A and FMTC. *Hum Mol Genet* 1993, 2(7):851-856. 42.Duronio V: The life of a cell: apoptosis regulation by the PI3K /PKB pathway. *Biochem J* 2008, 415(3):333-344. 43.El-Rayes BF, LoRusso PM: Targeting the epidermal growth factor receptor. *Br J Cancer* 2004, 91(3):418-424. 44.Fang WK, Gu W, Li EM, Wu ZY, Shen ZY, Shen JH, Wu JY, Pan F, Lv Z, Xu XE et al: Reduced membranous and ectopic cytoplasmic expression of DSC2 in esophageal squamous cell carcinoma: an independent prognostic factor. *Hum Pathol* 2010, 41(10):1456-1465. 45.Finlay CA, Hinds PW, Levine AJ: The p53 proto-oncogene can act as a suppressor of transformation. *Cell* 1989, 57(7):1083-1093. 46.Fishel R, Lescoe MK, Rao MR, Copeland NG, Jenkins NA, Garber J, Kane M, Kolodner R: The human mutator gene homolog MSH2 and its association with hereditary nonpolyposis colon cancer. *Cell* 1993, 75(5):1027-1038. 47.Folkman J: Angiogenesis and its inhibitors. *Important Adv Oncol* 1985:42-62. 48.Funakoshi S, Ezaki T, Kong J, Guo RJ, Lynch JP: Repression of the desmocollin 2 gene expression in human colon cancer cells is relieved by the homeodomain transcription factors Cdx1 and Cdx2. *Mol Cancer Res* 2008, 6(9):1478-1490. 49.Gao D, Nolan DJ, Mellick AS, Bambino K, McDonnell K, Mittal V:

Endothelial progenitor cells control the angiogenic switch in mouse lung metastasis. *Science* 2008, 319(5860):195-198. 50.Garber ME, Troyanskaya OG, Schluens K, Petersen S, Thaesler Z, Pacyna-Gengelbach M, van de Rijn M, Rosen GD, Perou CM, Whyte RI et al: Diversity of gene expression in adenocarcinoma of the lung. *Proc Natl Acad Sci U S A* 2001, 98(24):13784-13789. 51.Garrod D, Chidgey M: Desmosome structure, composition and function. *Biochim Biophys Acta* 2008, 1778(3):572-587. 52.Gehmlich K, Lambiase PD, Asimaki A, Ciaccio EJ, Ehler E, Syrris P, Saffitz JE, McKenna WJ: A novel desmocollin-2 mutation reveals insights into the molecular link between desmosomes and gap junctions. *Heart Rhythm* 2011, 8(5):711-718. 53.Getsios S, Huen AC, Green KJ: Working out the strength and flexibility of desmosomes. *Nat Rev Mol Cell Biol* 2004, 5(4):271-281. 54.Giampieri S, Pinner S, Sahai E: Intravital imaging illuminates transforming growth factor beta signaling switches during metastasis. *Cancer Res* 2010, 70(9):3435-3439. 55.Giannelli G, Bergamini C, Fransvea E, Sgarra C, Antonaci S: Laminin-5 with transforming growth factor-beta1 induces epithelial to mesenchymal transition in hepatocellular carcinoma. *Gastroenterology* 2005, 129(5):1375-1383. 56.Giannoni E, Bianchini F, Masieri L, Serni S, Torre E, Calorini L, Chiarugi P: Reciprocal activation of prostate cancer cells and cancer-associated fibroblasts stimulates epithelial-mesenchymal transition and cancer stemness. *Cancer Res* 2010, 70(17):6945-6956. 57.Giard DJ, Aaronson SA, Todaro GJ, Arnstein P, Kersey JH, Dosik H, Parks WP: In vitro cultivation of human tumors: establishment of cell lines derived from a series of solid tumors. *J Natl Cancer Inst* 1973, 51(5):1417-1423. 58.Glover KY, Perez-Soler R, Papadimitrakopoulou VA: A review of small-molecule epidermal growth factor receptor-specific tyrosine kinase inhibitors in development for non-small cell lung cancer. *Semin Oncol* 2004, 31(1 Suppl 1):83-92. 59.Goldman CK, Kim J, Wong WL, King V, Brock T, Gillespie GY: Epidermal growth factor stimulates vascular endothelial growth factor production by human malignant glioma cells: a model of glioblastoma multiforme pathophysiology. *Mol Biol Cell* 1993, 4(1):121-133. 60.Gorlova OY, Weng SF, Zhang Y, Amos CI, Spitz MR: Aggregation of cancer among relatives of never-smoking lung cancer patients. *Int J Cancer* 2007, 121(1):111-118. 61.Gotzmann J, Huber H, Thallinger C, Wolschek M, Jansen B, Schulte-Hermann R, Beug H, Mikulits W: Hepatocytes convert to a fibroblastoid phenotype through the cooperation of TGF-beta1 and Ha-Ras: steps towards invasiveness. *J Cell Sci* 2002, 115(Pt 6):1189-1202. 62.Green KJ, Simpson CL: Desmosomes: new perspectives on a classic. *J Invest Dermatol* 2007, 127(11):2499-2515. 63.Greenburg G, Hay ED: Epithelia suspended in collagen gels can lose polarity and express characteristics of migrating mesenchymal cells. *J Cell Biol* 1982, 95(1):333-339. 64.Grivaux M, Zureik M, Marsal L, Asselain B, Peureux M, Chavaillon JM, Prudhomme A, Carbonnelle M, Goarant E, Maury B et al: Five-year survival for lung cancer patients managed in general hospitals. *Rev Mal Respir* 2011, 28(7):e31-38. 65.Grusch M, Petz M, Metzner T, Ozturk D, Schneller D, Mikulits W: The crosstalk of RAS with the TGF-beta family during carcinoma progression and its implications for targeted cancer therapy. *Curr Cancer Drug Targets* 2010, 10(8):849-857. 66.Gupta PB, Onder TT, Jiang G, Tao K, Kuperwasser C, Weinberg RA, Lander ES: Identification of selective inhibitors of cancer stem cells by high-throughput screening. *Cell* 2009, 138(4):645-659. 67.Hackshaw AK, Law MR, Wald NJ: The accumulated evidence on lung cancer and environmental tobacco smoke. *BMJ* 1997, 315(7114):980-988. 68.Hamilton W, Peters TJ, Round A, Sharp D: What are the clinical features of lung cancer before the diagnosis is made? A population based case-control study. *Thorax* 2005, 60(12):1059-1065. 69.Han H, Bearss DJ, Browne LW, Calaluce R, Nagle RB, Von Hoff DD: Identification of differentially expressed genes in pancreatic cancer cells using cDNA microarray. *Cancer Res* 2002, 62(10):2890-2896. 70.Harris TJ, Tepass U: Adherens junctions: from molecules to morphogenesis. *Nat Rev Mol Cell Biol* 2010, 11(7):502-514. 71.Hartl M, Bader AG, Bister K: Molecular targets of the oncogenic transcription factor jun. *Curr Cancer Drug Targets* 2003, 3(1):41-55. 72.Hedenfalk IA, Ringner M, Trent JM, Borg A: Gene expression in inherited breast cancer. *Adv Cancer Res* 2002, 84:1-34. 73.Hegerfeldt Y, Tusch M, Brocker EB, Friedl P: Collective cell movement in primary melanoma explants: plasticity of cell-cell interaction, beta1-integrin function, and migration strategies. *Cancer Res* 2002, 62(7):2125-2130. 74.Herbst RS: Review of epidermal growth factor receptor biology. *Int J Radiat Oncol Biol Phys* 2004, 59(2 Suppl):21-26. 75.Heuser A, Plovie ER, Ellinor PT, Grossmann KS, Shin JT, Wichter T, Basson CT, Lerman BB, Sasse-Klaassen S, Thierfelder L et al: Mutant desmocollin-2 causes arrhythmogenic right ventricular cardiomyopathy. *Am J Hum Genet* 2006, 79(6):1081-1088. 76.Hippo Y, Taniguchi H, Tsutsumi S, Machida N, Chong JM, Fukayama M, Kodama T, Aburatani H: Global gene expression analysis of gastric cancer by oligonucleotide microarrays. *Cancer Res* 2002, 62(1):233-240. 77.Hirsch FR, Varella-Garcia M, Bunn PA, Jr., Di Maria MV, Veve R, Bremmes RM, Baron AE, Zeng C, Franklin WA: Epidermal growth factor receptor in non-small-cell lung carcinomas: correlation between gene copy number and protein expression and impact on prognosis. *J Clin Oncol* 2003, 21(20):3798-3807. 78.Holthofer B, Windoffer R, Troyanovsky S, Leube RE: Structure and function of desmosomes. *Int Rev Cytol* 2007, 264:65-163. 79.Hu T, Li C: Convergence between Wnt-beta-catenin and EGFR signaling in cancer. *Mol Cancer* 2010, 9:236. 80.Huang X, Saint-Jeannet JP: Induction of the neural crest and the opportunities of life on the edge. *Dev Biol* 2004, 275(1):1-11. 81.Hunter T, Cooper JA: Epidermal growth factor induces rapid tyrosine phosphorylation of proteins in A431 human tumor cells. *Cell* 1981, 24(3):741-752. 82.Ionov Y, Peinado MA, Malkhosyan S, Shibata D, Perucho M: Ubiquitous somatic mutations in simple repeated sequences reveal a new mechanism for colonic carcinogenesis. *Nature* 1993, 363(6429):558-561. 83.Jemal A, Bray F, Center MM, Ferlay J, Ward E, Forman D: Global cancer statistics. *CA Cancer J Clin* 2011, 61(2):69-90. 84.Jin T, George Fantus I, Sun J: Wnt and beyond Wnt: multiple mechanisms control the transcriptional property of beta-catenin. *Cell Signal* 2008, 20(10):1697-1704. 85.Kabir Z, Bennett K, Clancy L: Lung cancer and urban air-pollution in Dublin: a temporal association? *Ir Med J* 2007, 100(2):367-369. 86.Kalluri R, Neilson EG: Epithelial-mesenchymal transition and its implications for fibrosis. *J Clin Invest* 2003, 112(12):1776-1784. 87.Khan K, Hardy R, Haq A, Ogunbiyi O, Morton D, Chidgey M: Desmocollin switching in colorectal cancer. *Br J Cancer* 2006, 95(10):1367-1370. 88.Kljuic A, Bauer RC, Christiano AM: Genomic organization of mouse desmocollin genes reveals evolutionary conservation. *DNA Seq* 2004, 15(2):148-152. 89.Kolegraaff K, Nava P, Helms MN, Parkos CA, Nusrat A: Loss of desmocollin-2 confers a tumorigenic phenotype to colonic epithelial cells through activation of

Akt/{beta}-catenin signaling. *Mol Biol Cell* 2011, 22(8):1121-1134. 90.Kolegraaff K, Nava P, Helms MN, Parkos CA, Nusrat A: Loss of desmocollin-2 confers a tumorigenic phenotype to colonic epithelial cells through activation of Akt/beta-catenin signaling. *Mol Biol Cell* 2011, 22(8):1121-1134. 91.Kottke MD, Delva E, Kowalczyk AP: The desmosome: cell science lessons from human diseases. *J Cell Sci* 2006, 119(Pt 5):797-806. 92.Kowalczyk AP, Bornslaeger EA, Norvell SM, Palka HL, Green KJ: Desmosomes: intercellular adhesive junctions specialized for attachment of intermediate filaments. *Int Rev Cytol* 1999, 185:237-302. 93.Leach FS, Nicolaides NC, Papadopoulos N, Liu B, Jen J, Parsons R, Peltomaki P, Sistonen P, Aaltonen LA, Nystrom-Lahti M et al: Mutations of a mutS homolog in hereditary nonpolyposis colorectal cancer. *Cell* 1993, 75(6):1215-1225. 94.Lee JM, Dedhar S, Kalluri R, Thompson EW: The epithelial-mesenchymal transition: new insights in signaling, development, and disease. *J Cell Biol* 2006, 172(7):973-981. 95.Lehembre F, Yilmaz M, Wicki A, Schomber T, Strittmatter K, Ziegler D, Kren A, Went P, DerkSEN PW, Berns A et al: NCAM-induced focal adhesion assembly: a functional switch upon loss of E-cadherin. *EMBO J* 2008, 27(19):2603-2615. 96.Lemoine NR, Lobresco M, Leung H, Barton C, Hughes CM, Prigent SA, Gullick WJ, Kloppel G: The erbB-3 gene in human pancreatic cancer. *J Pathol* 1992, 168(3):269-273. 97.Li F, Ambrosini G, Chu EY, Plescia J, Tognin S, Marchisio PC, Altieri DC: Control of apoptosis and mitotic spindle checkpoint by survivin. *Nature* 1998, 396(6711):580-584. 98.Liu D, Aguirre Ghiso J, Estrada Y, Ossowski L: EGFR is a transducer of the urokinase receptor initiated signal that is required for in vivo growth of a human carcinoma. *Cancer Cell* 2002, 1(5):445-457. 99.Lorimer JE, Hall LS, Clarke JP, Collins JE, Fleming TP, Garrod DR: Cloning, sequence analysis and expression pattern of mouse desmocollin 2 (DSC2), a cadherin-like adhesion molecule. *Mol Membr Biol* 1994, 11(4):229-236. 100.Luo J, Dunn T, Ewing C, Sauvageot J, Chen Y, Trent J, Isaacs W: Gene expression signature of benign prostatic hyperplasia revealed by cDNA microarray analysis. *Prostate* 2002, 51(3):189-200. 101.Malkin D, Li FP, Strong LC, Fraumeni JF, Jr., Nelson CE, Kim DH, Kassel J, Gryka MA, Bischoff FZ, Tainsky MA et al: Germ line p53 mutations in a familial syndrome of breast cancer, sarcomas, and other neoplasms. *Science* 1990, 250(4985):1233-1238. 102.Mani SA, Guo W, Liao MJ, Eaton EN, Ayyanan A, Zhou AY, Brooks M, Reinhard F, Zhang CC, Shipitsin M et al: The epithelial-mesenchymal transition generates cells with properties of stem cells. *Cell* 2008, 133(4):704-715. 103.Markowitz S, Wang J, Myeroff L, Parsons R, Sun L, Lutterbaugh J, Fan RS, Zborowska E, Kinzler KW, Vogelstein B et al: Inactivation of the type II TGF-beta receptor in colon cancer cells with microsatellite instability. *Science* 1995, 268(5215):1336-1338. 104.Markowitz SD, Bertagnolli MM: Molecular origins of cancer: Molecular basis of colorectal cancer. *N Engl J Med* 2009, 361(25):2449-2460. 105.Massague J, Wotton D: Transcriptional control by the TGF-beta/Smad signaling system. *EMBO J* 2000, 19(8):1745-1754. 106.Matsuoka S, Huang M, Elledge SJ: Linkage of ATM to cell cycle regulation by the Chk2 protein kinase. *Science* 1998, 282(5395):1893-1897. 107.Mazzocca A, Fransvea E, Dituri F, Lupo L, Antonaci S, Giannelli G: Down-regulation of connective tissue growth factor by inhibition of transforming growth factor beta blocks the tumor-stroma cross-talk and tumor progression in hepatocellular carcinoma. *Hepatology* 2010, 51(2):523-534. 108.Moscatello DK, Holgado-Madruga M, Godwin AK, Ramirez G, Gunn G, Zoltick PW, Biegel JA, Hayes RL, Wong AJ: Frequent expression of a mutant epidermal growth factor receptor in multiple human tumors. *Cancer Res* 1995, 55(23):5536-5539. 109.Mulligan LM, Kwok JB, Healey CS, Elsdon MJ, Eng C, Gardner E, Love DR, Mole SE, Moore JK, Papi L et al: Germ-line mutations of the RET proto-oncogene in multiple endocrine neoplasia type 2A. *Nature* 1993, 363(6428):458-460. 110.Nagai Y, Miyazawa H, Huqun, Tanaka T, Udagawa K, Kato M, Fukuyama S, Yokote A, Kobayashi K, Kanazawa M et al: Genetic heterogeneity of the epidermal growth factor receptor in non-small cell lung cancer cell lines revealed by a rapid and sensitive detection system, the peptide nucleic acid-locked nucleic acid PCR clamp. *Cancer Res* 2005, 65(16):7276-7282. 111.Neilson EG: Setting a trap for tissue fibrosis. *Nat Med* 2005, 11(4):373-374. 112.Nishida T, Hirota S, Taniguchi M, Hashimoto K, Isozaki K, Nakamura H, Kanakura Y, Tanaka T, Takabayashi A, Matsuda H et al: Familial gastrointestinal stromal tumours with germline mutation of the KIT gene. *Nat Genet* 1998, 19(4):323-324. 113.Nolan DJ, Ciarrocchi A, Mellick AS, Jaggi JS, Bambino K, Gupta S, Heikamp E, McDevitt MR, Scheinberg DA, Benzeira R et al: Bone marrow-derived endothelial progenitor cells are a major determinant of nascent tumor neovascularization. *Genes Dev* 2007, 21(12):1546-1558. 114.Nollet F, Kools P, van Roy F: Phylogenetic analysis of the cadherin superfamily allows identification of six major subfamilies besides several solitary members. *J Mol Biol* 2000, 299(3):551-572. 115.Oda K, Matsuoka Y, Funahashi A, Kitano H: A comprehensive pathway map of epidermal growth factor receptor signaling. *Mol Syst Biol* 2005, 1:2005 0010. 116.O'Reilly KM, McLaughlin AM, Beckett WS, Sime PJ: Asbestos-related lung disease. *Am Fam Physician* 2007, 75(5):683-688. 117.Osaki M, Oshimura M, Ito H: PI3K-Akt pathway: its functions and alterations in human cancer. *Apoptosis* 2004, 9(6):667-676. 118.Parker AE, Wheeler GN, Arnemann J, Pidsley SC, Ataliotis P, Thomas CL, Rees DA, Magee AI, Buxton RS: Desmosomal glycoproteins II and III. Cadherin-like junctional molecules generated by alternative splicing. *J Biol Chem* 1991, 266(16):10438-10445. 119.Parker C, Roseman BJ, Bucana CD, Tsan R, Radinsky R: Preferential activation of the epidermal growth factor receptor in human colon carcinoma liver metastases in nude mice. *J Histochem Cytochem* 1998, 46(5):595-602. 120.Peinado H, Olmeda D, Cano A: Snail, Zeb and bHLH factors in tumour progression: an alliance against the epithelial phenotype? *Nat Rev Cancer* 2007, 7(6):415-428. 121.Perry JE, Grossmann ME, Tindall DJ: Epidermal growth factor induces cyclin D1 in a human prostate cancer cell line. *Prostate* 1998, 35(2):117-124. 122.Pesch B, Kendzia B, Gustavsson P, Jockel KH, Johnen G, Pohlbeln H, Olsson A, Ahrens W, Gross IM, Broske I et al: Cigarette smoking and lung cancer - relative risk estimates for the major histological types from a pooled analysis of case-control studies. *Int J Cancer* 2011. 123.Plowman GD, Culouscou JM, Whitney GS, Green JM, Carlton GW, Foy L, Neubauer MG, Shoyab M: Ligand-specific activation of HER4/p180erbB4, a fourth member of the epidermal growth factor receptor family. *Proc Natl Acad Sci U S A* 1993, 90(5):1746-1750. 124.Pokutta S, Weis WI: Structure and mechanism of cadherins and catenins in cell-cell contacts. *Annu Rev Cell Dev Biol* 2007, 23:237-261. 125.Polakis P: The many ways of Wnt in cancer. *Curr Opin Genet Dev* 2007, 17(1):45-51. 126.Prakash I, Mathur RP, Kar P, Ranga

S, Talib VH: Comparative evaluation of cell proliferative indices and epidermal growth factor receptor expression in gastric carcinoma. Indian J Pathol Microbiol 1997, 40(4):481-490. 127.Prenzel N, Zwick E, Daub H, Leserer M, Abraham R, Wallasch C, Ullrich A: EGF receptor transactivation by G-protein-coupled receptors requires metalloproteinase cleavage of proHB-EGF. Nature 1999, 402(6764):884-888. 128.Price JT, Wilson HM, Haites NE: Epidermal growth factor (EGF) increases the in vitro invasion, motility and adhesion interactions of the primary renal carcinoma cell line, A704. Eur J Cancer 1996, 32A(11):1977-1982. 129.Radinsky R, Risin S, Fan D, Dong Z, Bielenberg D, Bucana CD, Fidler IJ: Level and function of epidermal growth factor receptor predict the metastatic potential of human colon carcinoma cells. Clin Cancer Res 1995, 1(1):19-31. 130.Raz DJ, He B, Rosell R, Jablons DM: Bronchioloalveolar carcinoma: a review. Clin Lung Cancer 2006, 7(5):313-322. 131.Rosen EM, Goldberg ID: Protein factors which regulate cell motility. In Vitro Cell Dev Biol 1989, 25(12):1079-1087. 128.Rosti G, Bevilacqua G, Bidoli P, Portalone L, Santo A, Genestreti G: Small cell lung cancer. Ann Oncol 2006, 17 Suppl 2:ii5-10. 132.Russo G, Zegar C, Giordano A: Advantages and limitations of microarray technology in human cancer. Oncogene 2003, 22(42):6497-6507. 133.Salomon DS, Brandt R, Ciardiello F, Normanno N: Epidermal growth factor-related peptides and their receptors in human malignancies. Crit Rev Oncol Hematol 1995, 19(3):183-232. 134.Schmidt L, Duh FM, Chen F, Kishida T, Glenn G, Choyke P, Scherer SW, Zhuang Z, Lubensky I, Dean M et al: Germline and somatic mutations in the tyrosine kinase domain of the MET proto-oncogene in papillary renal carcinomas. Nat Genet 1997, 16(1):68-73. 135.Shibata T, Kawano T, Nagayasu H, Okumura K, Arisue M, Hamada J, Takeichi N, Hosokawa M: Enhancing effects of epidermal growth factor on human squamous cell carcinoma motility and matrix degradation but not growth. Tumour Biol 1996, 17(3):168-175. 136.Shinohara M, Hiraki A, Ikebe T, Nakamura S, Kurahara S, Shirasuna K, Garrod DR: Immunohistochemical study of desmosomes in oral squamous cell carcinoma: correlation with cytokeratin and E-cadherin staining, and with tumour behaviour. J Pathol 1998, 184(4):369-381. 137.Simon AM, Goodenough DA: Diverse functions of vertebrate gap junctions. Trends Cell Biol 1998, 8(12):477-483. 138.Sobol RE, Astarita RW, Hofeditz C, Masui H, Fairshter R, Royston I, Mendelsohn J: Epidermal growth factor receptor expression in human lung carcinomas defined by a monoclonal antibody. J Natl Cancer Inst 1987, 79(3):403-407. 139.Sordella R, Bell DW, Haber DA, Settleman J: Gefitinib-sensitizing EGFR mutations in lung cancer activate anti-apoptotic pathways. Science 2004, 305(5687):1163-1167. 140.Strand TE, Rostad H, Damhuis RA, Norstein J: Risk factors for 30-day mortality after resection of lung cancer and prediction of their magnitude. Thorax 2007, 62(11):991-997. 141.Subramanian J, Govindan R: Lung cancer in never smokers: a review. J Clin Oncol 2007, 25(5):561-570. 142.Sugarbaker DJ, Dasilva MC: Diagnostic workup of lung cancer. Surg Oncol Clin N Am 2011, 20(4):667-679. 143.Syrris P, Ward D, Evans A, Asimaki A, Gandjbakhch E, Sen-Chowdhry S, McKenna WJ: Arrhythmogenic right ventricular dysplasia cardiomyopathy associated with mutations in the desmosomal gene desmocollin-2. Am J Hum Genet 2006, 79(5):978-984. 144.Tamm I, Wang Y, Sausville E, Scudiero DA, Vigna N, Oltersdorf T, Reed JC: IAP-family protein survivin inhibits caspase activity and apoptosis induced by Fas (CD95), Bax, caspases, and anticancer drugs. Cancer Res 1998, 58(23):5315-5320. 145.Tauler J, Zudaire E, Liu H, Shih J, Mulshine JL: hnRNP A2/B1 modulates epithelial-mesenchymal transition in lung cancer cell lines. Cancer Res 2010, 70(18):7137-7147. 146.Temme A, Rieger M, Reber F, Lindemann D, Weigle B, Diestelkoetter-Bachert P, Ehninger G, Tatsuka M, Terada Y, Rieber EP: Localization, dynamics, and function of survivin revealed by expression of functional survivinDsRed fusion proteins in the living cell. Mol Biol Cell 2003, 14(1):78-92. 147.Thiery JP: Epithelial-mesenchymal transitions in tumour progression. Nat Rev Cancer 2002, 2(6):442-454. 148.Thiery JP, Acloque H, Huang RY, Nieto MA: Epithelial-mesenchymal transitions in development and disease. Cell 2009, 139(5):871-890. 149.Thun MJ, Hannan LM, Adams-Campbell LL, Boffetta P, Buring JE, Feskanich D, Flanders WD, Jee SH, Katanoda K, Kolonel LN et al: Lung cancer occurrence in never-smokers: an analysis of 13 cohorts and 22 cancer registry studies. PLoS Med 2008, 5(9):e185. 150.Tonin PN, Hudson TJ, Rodier F, Bossolasco M, Lee PD, Novak J, Manderson EN, Provencher D, Mes-Masson AM: Microarray analysis of gene expression mirrors the biology of an ovarian cancer model. Oncogene 2001, 20(45):6617-6626. 151.Tracy S, Mukohara T, Hansen M, Meyerson M, Johnson BE, Janne PA: Gefitinib induces apoptosis in the EGFR-L858R non-small-cell lung cancer cell line H3255. Cancer Res 2004, 64(20):7241-7244. 152.Travis WD: Pathology of lung cancer. Clin Chest Med 2002, 23(1):65-81, viii. 153.Travis WD, Travis LB, Devesa SS: Lung cancer. Cancer 1995, 75(1 Suppl):191-202. 154.Tselepis C, Chidgey M, North A, Garrod D: Desmosomal adhesion inhibits invasive behavior. Proc Natl Acad Sci U S A 1998, 95(14):8064-8069. 155.Turner T, Chen P, Goodly LJ, Wells A: EGF receptor signalling enhances in vivo invasiveness of DU-145 human prostate carcinoma cells. Clin Exp Metastasis 1996, 14(4):409-418. 156.Uren AG, Wong L, Pakusch M, Fowler KJ, Burrows FJ, Vaux DL, Choo KH: Survivin and the inner centromere protein INCENP show similar cell-cycle localization and gene knockout phenotype. Curr Biol 2000, 10(21):1319-1328. 157.Veale D, Ashcroft T, Marsh C, Gibson GJ, Harris AL: Epidermal growth factor receptors in non-small cell lung cancer. Br J Cancer 1987, 55(5):513-516. 158.Velculescu VE, Zhang L, Vogelstein B, Kinzler KW: Serial analysis of gene expression. Science 1995, 270(5235):484-487. 159.Verbeek BS, Adriaansen-Slot SS, Vroom TM, Beckers T, Rijken G: Overexpression of EGFR and c-erbB2 causes enhanced cell migration in human breast cancer cells and NIH3T3 fibroblasts. FEBS Lett 1998, 425(1):145-150. 160.Villeneuve PJ, Mao Y: Lifetime probability of developing lung cancer, by smoking status, Canada. Can J Public Health 1994, 85(6):385-388. 161.Vincent-Salomon A, Thiery JP: Host microenvironment in breast cancer development: epithelial-mesenchymal transition in breast cancer development. Breast Cancer Res 2003, 5(2):101-106. 162.Vlahovic G, Crawford J: Activation of tyrosine kinases in cancer. Oncologist 2003, 8(6):531-538. 163.von Eyben FE: Epidermal growth factor receptor inhibition and non-small cell lung cancer. Crit Rev Clin Lab Sci 2006, 43(4):291-323. 164.Wahl MI, Carpenter G: Role of growth factors and their receptors in the control of normal cell proliferation and cancer. Clin Physiol Biochem 1987, 5(3-4):130-139. 165.Wang Q, Greene MI: EGFR enhances Survivin expression through the phosphoinositide 3 (PI-3) kinase signaling pathway. Exp Mol Pathol 2005, 79(2):100-107. 166.Watanabe H: [Extracellular matrix--regulation of cancer invasion and metastasis]. Gan To Kagaku Ryoho 2010,

37(11):2058-2061. 167.Wolf K, Mazo I, Leung H, Engelke K, von Andrian UH, Deryugina EI, Strongin AY, Brocker EB, Friedl P: Compensation mechanism in tumor cell migration: mesenchymal-amoebooid transition after blocking of pericellular proteolysis. *J Cell Biol* 2003, 160(2):267-277.

168.Woodburn JR: The epidermal growth factor receptor and its inhibition in cancer therapy. *Pharmacol Ther* 1999, 82(2-3):241-250. 169.Yarden Y: The EGFR family and its ligands in human cancer. signalling mechanisms and therapeutic opportunities. *Eur J Cancer* 2001, 37 Suppl 4:S3-8.

170.Yasui W, Sumiyoshi H, Hata J, Kameda T, Ochiai A, Ito H, Tahara E: Expression of epidermal growth factor receptor in human gastric and colonic carcinomas. *Cancer Res* 1988, 48(1):137-141. 171.Yilmaz M, Christofori G: Mechanisms of motility in metastasizing cells. *Mol Cancer Res* 2010, 8(5):629-642. 172.Yonemura Y, Takamura H, Ninomiya I, Fushida S, Tsugawa K, Kaji M, Nakai Y, Ohoyama S, Yamaguchi A, Miyazaki I: Interrelationship between transforming growth factor-alpha and epidermal growth factor receptor in advanced gastric cancer. *Oncology* 1992, 49(2):157-161. 173.Yoshida BA, Sokoloff MM, Welch DR, Rinker-Schaeffer CW: Metastasis-suppres