Journal of Cellular Biochemistry 104:2071–2077 (2008)

AKT as Locus of Fragility in Robust Cancer System

Ziv Radisavljevic*

Department of Surgery, Brigham and Women's Hospital, Harvard Medical School, Boston, Massachusetts 02115

Abstract Metastatic cancer is a complex positive feedback loop system. Such as system has a tendency to acquire extreme robustness. Signaling pathways controlling that robustness can fail completely if an essential element from the signaling is removed. That element is a locus of fragility. Targeting that locus represents the best way to target the cancer robustness. This prospect presents another locus of fragility in signaling complex system network, controlling the cell cycle progression through the PI3K/AKT/mTOR/RAN pathway and cell migration and angiogenesis through the VEGF/PI3K/AKT/NO/ICAM-1 pathway. The locus of fragility of these pathways is AKT, which is regulated by a balance of catalase/H_2O_2 or by AKT inhibitor. Tiny and trivial perturbations such as change in redox state in the cells by antioxidant enzyme catalase, scavenging H_2O_2 signaling molecule, regulates robust signaling molecule AKT, abolishing its phosporilation and inducing cascading failure of robust signaling pathways for cell growth, proliferation, migration, and angiogenesis. An anticancer effect of the antioxidant is achieved through the AKT locus, by abolishing signals from growth factors VEGF, HGF, HIF-1α and H_2O_2. Previously reported locus of fragility nitric oxide (NO) and locus AKT are close in the complex signaling interactome network, but they regulate distinct signaling modules. Simultaneously targeted loci represents new principles in cancer robustness chemotherapy by blocking cell proliferation, migration, angiogenesis and inducing rather slow then fast apoptosis leading to slow eradication of cancer. J. Cell. Biochem. 104: 2071–2077, 2008.

Key words: AKT; locus of fragility; cancer; signaling; complex robust system

A cancer is a complex and a very robust system [Radisavljevic, 2004a]. A cancer cells acquire great robustness during the metastatic progression. Cellular signal transduction network are robust system [Barkai and Leibler, 1997]. Signaling mechanisms which regulate cancer phenotype are crucial for controlling robustness. Locus of fragility is the best way to target robustness [Radisavljevic, 2004a].

COMPLEXITY AND ROBUSTNESS OF CANCER CELL SYSTEM

Robustness is maintenance of the functional system characteristics despite fluctuations of its component parts and it is achieved through internal complexity of the system [Carlson and Doyle, 2002]. Statistical physics define robustness as an error tolerant system or a system insensitive to large variations. Even failure of components can be tolerated if they are regulated by redundancy feedback in the complex system [Carlson and Doyle, 1999, 2002]. High optimized tolerance (HOT) theory from statistical physics is the conceptual framework to study fundamental aspects of complexity [Carlson and Doyle, 1999, 2002]. The HOT system has high performance, high structural internal complexity with high densities of the interaction, simple robust external behavior and reliability, with the risk to potentially cascading failure initiated by possibly quite small trivial perturbations [Carlson and Doyle, 1999, 2002;

Abbreviations used: EGF, epidermal growth factor; FASN, fatty acid synthase; HGF, hepatocyte growth factor; HIF-1α, hypoxia inducible factor 1 alpha; HOT, high optimized tolerance theory; ICAM-1, intracellular adhesion molecule 1; mTOR, mammalian target of rapamycin; NO, nitric oxide; NOS, nitric oxide synthase; NuMA, nuclear mitotic apparatus protein; PI3K, phosphoinositol-3-OH kinase; PKB, protein kinase B; RAN, ras-related nuclear protein; ROS, reactive oxygen species; VEGF, vascular endothelial growth factor; VHL, Von Hippel–Lindau.

Grant sponsor: Yad Chessed Foundation.

*Correspondence to: Dr. Ziv Radisavljevic, MD, PhD, Department of Surgery, Brigham and Women's Hospital, Harvard Medical School, 75 Francis Street, Boston, MA 02115. E-mail: zradisavljevic@rics.bwh.harvard.edu

Received 6 March 2008; Accepted 10 March 2008

DOI 10.1002/jcb.21777

Zhou et al., 2002]. HOT theory postulated that system which acquires robustness against a common perturbation tends to be extremely fragile to some unexpected, tiny (trivial) perturbation [Carlson and Doyle, 1999, 2002; Csete and Doyle, 2002]. HOT power law presents complex system as robust, yet fragile [Carlson and Doyle, 1999, 2002]. In complex systems feedback control is both a powerful and dangerous for creating robustness to external disturbances and internal component variations [Csete and Doyle, 2002].

Robustness of cancer systems are built from a protein–protein or gene–gene interaction with positive feedback, that leads to creation of the system with extreme robustness. If essential component of the signaling system is targeted, system can be disabled by cascading failure initiated by tiny (trivial) perturbations. This essential component of the system represents locus of fragility in the cancer robust system [Radisavljevic, 2004a]. Locus of fragility in the breast cancer robust system was recently introduced as a first experimental confirmation of the HOT power law complex system, which if targeted by small perturbations induced failure of all signaling interconnecting pathways in the robust system [Radisavljevic, 2004a]. This prospect has tendency to use HOT theory as an excellent mathematical model to explain cancer cellular functional complexity and robustness and to present another locus of fragility in the complex cancer signaling system as AKT. If AKT locus is targeted by a small perturbation of redox state or by AKT inhibition, a cell cycle arrest, an abolishment of cell migration and angiogenesis occur, the components extremely important in the metastatic cancer progression.

ROBUSTNESS AND FRAGILITY OF SIGNALING PATHWAYS

Cell cycle progression, cell migration and angiogenesis are main component of the cancer metastatic progression [Radisavljevic, 2004a]. These cellular processes are regulated by complex robust signaling network [Radisavljevic et al., 2000; Radisavljevic, 2003, 2004a,b; Radisavljevic and Gonzalez-Flecha, 2003, 2004], but they are extremely fragile to tiny (trivial) perturbation if locus of fragility is targeted and disrupted in that system inducing failure of cancer robustness [Radisavljevic, 2004a]. The locus of fragility of the robust breast cancer system was recently introduced as a basic concept how to target cancer robustness [Radisavljevic, 2004a]. When locus of fragility was targeted by trivial perturbation (suppression of signaling molecule nitric oxide-NO) inhibiting NOS enzyme, failure of cell cycle progression occurs, abolishment of cell migration and angiogenesis and induction of slow apoptosis of cancer cells occur through the NO/ROCK/FOXO3a signaling pathway [Radisavljevic, 2003, 2004a]. This is new approach for robust metastatic cancer chemotherapy. In this prospect another locus of fragility is presented which is protein kinase B (PKB), known also as AKT molecule.

A feedback control of the complex system function is the most critical for establishing robustness. Once activated signaling processes in the metastatic cancer, get more powerful with magnificent amplification of the signals and signaling get very robust without negative feedback loop with speedy cumulative pattern. Furthermore, creation of the positive feedback loop occurs. The system responds in the same direction as stimulation and the positive signals create extreme robustness of the cancer through the amplified gene transcriptions and protein translation of the components inside of signaling pathways. The positive feedback loop system have tendency to acquire extreme robustness such as in the metastatic cancer. Targeting locus of fragility can bring robustness under control.

Cell cycle progression is driven by the PI3K/AKT/mTOR/RAN pathway [Radisavljevic and Gonzalez-Flecha, 2004]. On the other hand, cell migration and angiogenesis by the VEGF/PI3K/AKT/NO/ICAM-1 pathway [Radisavljevic et al., 2000]. The robustness of these signaling pathways was abolished by catalase, an antioxidant enzyme that scavenges H_2O_2, or by AKT inhibitor wortmannin, which prevents phosphorilation of AKT ser 473 (Fig. 1). The balance of the catalase/H_2O_2 is super class regulator, or master regulator of the phosphorylated AKT, an active form of protein kinase B (AKT), which is critical for the signaling integrity of the pathways in the cell migration, the angiogenesis and the cell proliferation. The AKT ser 473 phosphorylation induced by transfection of the constitutively active myr-AKT (mA) (pLNCX-HA-myr-AKT) or induced by the system glucose/glucose oxidase/catalase (S) generated signaling molecule H_2O_2 [Radisavljevic et al., 2000;

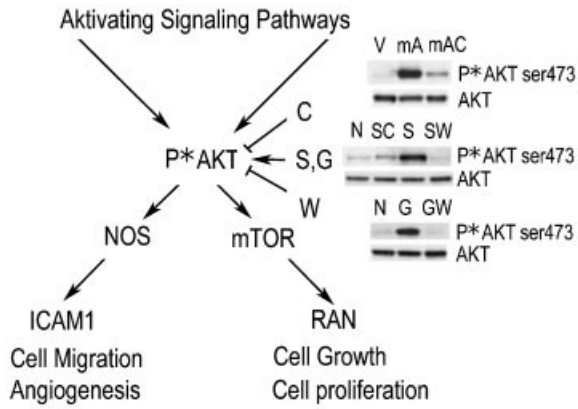

Fig. 1. AKT locus of fragility in robust cancer signaling. The cell growth and the cell proliferation are controlled via the PI3K/AKT/mTOR/RAN pathway, and the angiogenesis is controlled through the VEGF/PI3K/AKT/NO/ICAM1 pathway. The locus of fragility in these signaling systems is the AKT. The trivial perturbation such as change in redox state by an antioxidant enzyme catalase 300 nM (C), or AKT-inhibitor wortmannin 100 nM (W) abolished phosphorylation and activation of AKT and induced failure of the robust signaling pathways (Western blot of the A549 cancer cell line from ATCC; 100 mg of total cell lysate was resolved by 8% SDS–polyacrylamide gel electrophoresis). Vector pLNCX (V), constitutively active myr-AKT-HA (mA), control non treated (N), growth factor (G) $VEGF_{165}$ 100 ng/ml, catalase/glucose oxidase system generated H_2O_2 powerful signaling molecule (S) (glucose oxidase 20 mU/ml with catalase 300 nM in medium), phosphorylated AKT ser-473 (P*AKT) (polyclonal antibodies from Cell Signaling, Beverly, MA). Activating signals are growth factors such as: HGF, VEGF, HIF-1α, and the H_2O_2 of activating range.

Radisavljevic and Gonzalez-Flecha, 2004], was abolished by catalase (C) (myr-AKT plus catalase-mAC, or glucose/glucose oxidase/catalase system plus catalase-SC), and also by AKT inhibitor wortmannin (W), indicating that signals cannot go through without active AKT. This confirms that AKT is the locus of fragility, and that, the balance of the catalase/H_2O_2 is master regulator of the active AKT (Fig. 1). An enzymatic model consisting of glucose/glucose oxidase and catalase together, that allows the controlled generation of H_2O_2 mimics the oxidative burst induced in physiological condition, where H_2O_2 signaling molecule freely diffuses from the medium into the cell inducing AKT phosphorilation and activating the cell proliferation [Radisavljevic and Gonzalez-Flecha, 2004]. H_2O_2 signaling molecule from that system alone was able to induce AKT phosphorylation and addition of catalase was able to abolish that phosphorylation. If only glucose/glucose oxidase or H_2O_2 was used, activation of apoptotic pathway occurs [Radisavljevic and Gonzalez-Flecha, 2003]. For activation of the cell proliferation pathways H_2O_2 is required above physiological, but bellow apoptotic concentration [Radisavljevic and Gonzalez-Flecha, 2003]. Upper physiological range of the H_2O_2 activates the key signaling molecule AKT [Radisavljevic and Gonzalez-Flecha, 2003, 2004].

Recently was reported [Semenza, 2003] that hypoxia inducible factor alpha 1 (HIF-1α) is a master regulator of clusters of genes in the cancer, but appears that, with this prospect, antioxidant enzyme catalase and H_2O_2 signaling molecule are super master regulators of HIF-1α, that regulate active form of AKT together with its specific inhibitor wortmannin, and because HIF-1α signaling is regulated through the AKT [Lee et al., 2008] indicating that AKT is master regulator of these clusters of genes in the chain reaction manner from catalase/H_2O_2 balance to the AKT. The experimental facts in this prospect also challenge the paradigm that antitumorigenic effect of antioxidants is dependent on the HIF-1 [Gao et al., 2007], but HIF-1α is regulated by AKT [Lee et al., 2008], indicating that an anticancer effect of the antioxidant is achieved through AKT locus not through the HIF-1α. The super regulator of phosphorylated AKT is the catalase/H_2O_2 balance system or AKT inhibitor wortmannin. AKT represents locus of fragility in the cancer robust signaling system. In solid tumors such as medulloblastoma AKT pathway regulates survival of a cancer stem cells residing in the perivascular niche after radiation [Hambardzumyan et al., 2008]. Also, recently was reported that the fatty acid synthase (FASN) gene is up-regulated in various types of cancers by HIF1 released during hypoxia and that reactive oxygen species (ROS) like H_2O_2 activates AKT. The conclusion in that report was made that the FASN represents an attractive target for anticancer therapy [Furuta et al., 2008]. But, here I am presenting AKT as essential locus of fragility for targeting cancer robustness by trivial perturbation such as redox state using antioxidant enzyme catalase or AKT inhibitor, in very simplistic, trivial but very effective way.

AKT Is Locus of Fragility in Cell Proliferation

Activation of the serine/threonine protein kinase AKT/PKB by phosphorylation mediates activation of the signaling pathways leading toward cell growth and cell proliferation through the PI3K/AKT/mTOR/RAN pathway

[Radisavljevic and Gonzalez-Flecha, 2004]. Cell growth, an increase in cell mass is a result of an enhanced synthesis of proteins [Tapon et al., 2001] and is mediated by AKT and the mammalian target of rapamycin (mTOR) [Schmelzle and Hall, 2000; Scheidenhelm et al., 2005; Krishnan et al., 2006]. On the other hand, cell proliferation (cell cycle progression) resulting in cell division and increase in cell number is controlled also by AKT and mTOR [Radisavljevic and Gonzalez-Flecha, 2004]. Cell proliferation requires cell growth, however, these two processes are distinct but they are mediated by the same signaling molecules AKT/mTOR. The growth factors, such as an epidermal growth factor (EGF), a vascular endothelial growth factor (VEGF), HIF-1α, and angiotensin II induce H_2O_2 production and AKT phosphorylation in ovarian cancer cell proliferation [Gao et al., 2002, 2004; Liu et al., 2006] and prostate cancer cells proliferation [Uemura et al., 2008]. The growth factors initiated their activity through ROS burst, a H_2O_2 signaling molecule, which activates AKT signaling [Radisavljevic and Gonzalez-Flecha, 2004; Liu et al., 2006; Uemura et al., 2008]. Also, the active AKT promotes the cell growth in acute myeloid leukemia (AML) [Tazzari et al., 2004], and in a chronic myeloid leukemia (CML) [Van Etten, 2007] and contribute to genesis of sarcoma [Sodhi et al., 2004].

Nucleocytoplasmic trafficking and spindle pole assembly are critical steps in the control of cell proliferation [Percipalle et al., 1999]. These processes are regulated by active AKT and ROS such as H_2O_2 and has enormous role in mediating this process [Radisavljevic and Gonzalez-Flecha, 2004]. The RAN, a small guanine nucleotide triphosphatase (GTPase) is required for nucleocytoplasmatic transport and for the induction of spindle assembly [Smith et al., 2002] as well as post-mitotic nuclear assembly [Dasso, 2001]. The RAN regulates nuclear import and export processes through nuclear the pore complexes and importin-alpha, inside of imporin alpha–beta complex [Gruss et al., 2001] and NuMA, which regulates the balance of microtubule-motor activities during spindle assembly [Wilde et al., 2001], both are regulated by H_2O_2 signaling molecule and antioxidant enzyme catalase [Radisavljevic and Gonzalez-Flecha, 2003, 2004] through the AKT locus.

Antioxidant enzyme catalase is present mainly in the peroxisomes of the cell. Catalase is a tetrameric protein with four identical subunits of 527 amino acid (60 kDa), heme group and NADPH molecule [Chelikani et al., 2003]. The catalase reaction utilizes hydrogen peroxide (H_2O_2) as both an electron donor and an electron acceptor [Chelikani et al., 2003]. Catalase acts catalytically to remove H_2O_2 by forming H_2O and O_2 if the concentration of H_2O_2 is high. At a low H_2O_2 concentration in presence of a hydrogen donor such as ethanol, methanol, phenol, catalase acts peroxidically by removing H_2O_2 and oxidizing its substrate (peroxidatic reaction). Active site of catalase, a catalytic domain is conserved domain located in the hydrophobic core of protein in the length of 30–50 Å [Chelikani et al., 2003]. A number of highly conserved residues are situated in the main catalytic channel including the essential histidine, valine and aspartate (His128, Val169, and Asp181) situated 4, 8, and 12 Å from a heme. The His128 is essential for catalysis and the importance of Val169 in constricting the narrowest, hydrophobic portion of the channel. In the main channel Glu181 is negatively charged and the heme is positively charged. The potential between these two charges on the electrical dipoles of water in the channel and active site represent main force for puling H_2O_2 into channel for degradation [Chelikani et al., 2003].

Cell proliferation is regulated through the PI3K/AKT/mTOR/RAN pathway [Radisavljevic and Gonzalez-Flecha, 2004], and H_2O_2 initiated this signaling process by phosphorilating AKT ser-473 site, but catalase eliminating H_2O_2, abolished phosphorilation of AKT and all signaling pathway and cell proliferation failed (Fig. 1). The RAN cargo macromolecule is upregulated in cell cycle progression, but all process is abolished by targeting AKT with antioxidant enzyme catalase which removes H_2O_2 and prevents AKT activation. The AKT represent locus of fragility of this robust sell proliferation signaling pathway.

AKT Is Locus of Fragility in Cell Migration and Angiogenesis

A phosphorylated and an activated AKT/PKB has crucial role in mediating cell migration and angiogenesis [Radisavljevic et al., 2000]. A VEGF activates AKT in signaling cascade leading to the cell migration in cancer angiogenesis [Radisavljevic, 2004a]. The VEGF is a key angiogenic molecule in many types of

human metastatic cancers [Shi et al., 2001]. Hypoxia is a key regulatory factor in cancer growth [Harris, 2002]. Hypoxia helps cancer cells undergo genetic and adaptive changes that allow them to survive and even proliferate in the hypoxic environment. These processes contribute to the malignant phenotype and to aggressive tumor behavior [Harris et al., 2002]. In tumor area distant from microvessels where hypoxia is present, HIF-1α is present in high concentration. In hypoxia the HIF-1α translocates to the nucleus and dimerizes with HIF-1β to form HIF1. The HIF1 binds to regulatory regions of target genes such as the VEGF [Mazure et al., 1996] and hepatocyte growth factors (HGF) up-regulating their expression [Trusolino and Comoglio, 2002]. The HGF and hypoxia activate synergistically c-met elevating cancer cell motility and invasion [Steeg, 2003; Pennacchietti et al., 2003]. This makes HIF1 the master regulator of oxygen homeostasis to meet cell and tissue requirements in a situation of oxygen deficiency [Wang et al., 1995]. In the presence of oxygen (normoxia), HIF-1α is hydroxylated at Pro-564 and Pro-402 by prolyl hydroxylase, an enzyme which covalently modifies HIF-1α and converting it to a hydroxylated form and bound to the tumor suppressor Von Hippel–Lindau (VHL) protein, ubiquitylated and degraded in the proteasome [Masson et al., 2001]. HIF-1α is overexpressed in colon, breast, gastric, lung, skin, ovarian, pancreatic, renal, and prostate cancer and associated with cell proliferation [Harris, 2002]. Hypoxia driven generation of VEGF [Mazure et al., 1996] up-regulates intracellular adhesion molecule-1 (ICAM1) synergistically with L-arginine, a substrate for the nitric oxide synthase (NOS) through the phosphorylated AKT ser 473 [Radisavljevic et al., 2000]. The AKT specific inhibitor wortmannin abolished cell migration induced by the VEGF and nitric oxide through the ICAM1, an effector signaling molecule in cell migration and angiogenesis by inactivating the VEGF/PI3K/AKT/NO/ICAM-1 signaling pathway. The AKT is locus of fragility in this robust signaling system, which can be targeted either by catalase or by PI3K/AKT inhibitor (Fig. 1).

CONCLUDING REMARKS

Metastatic cancer is a complex system with a positive feedback loop. Such a system has tendency to acquire extreme robustness, and negative feedback loop is a very difficult to establish. Targeting locus of fragility in that extreme robustness can induce system failure. Signaling mechanisms which control cancer responses are crucial for the controlling robustness. Identification of locus of fragility in cancer represents basic mechanism to target robustness. Complex cellular signaling system gets robust by activation of interacting signaling pathways. Each gene and protein participates as a single component in signaling pathways in building robustness. When signal transduction is activated it integrates all components in very complex and robust system. The positive feedback loops in the metastatic cancer are creating an extreme robustness. The main dilemma in Oncology is how to approach that robustness. The AKT represents a locus of fragility in robust signaling pathways, which regulates the cell proliferation, the cell migration and the angiogenesis, if targeted by the antioxidant enzyme catalase, a trivial perturbation of the redox state (reductive/oxidative reaction) occurs, and all signaling and robustness fail. Anti-cancer effect of the antioxidant is achieved by dephosphorylation of the AKT signaling locus. Previously reported locus of fragility NOS if simultaneously targeted with AKT locus great effect can be achieved in treatment of malignant metastatic diseases. This new principle of simultaneous approach can abolish malignant cell robustness, by blocking cell migration, angiogenesis, cell cycle progression and inducing slow apoptosis and initiating cascading failure of robustness of the cancer. These two loci of fragility represent experimental confirmation of the HOT power law system, which is robust, but fragile.

ACKNOWLEDGMENTS

This work was supported by Yad Chessed Foundation.

REFERENCES

Barkai N, Leibler S. 1997. Robustness in simple biochemical networks. Nature 387:913–917.

Carlson JM, Doyle J. 1999. Highly optimized tolerance: A mechanism for power laws in designed systems. Phys Rev E Stat Phys Plasmas Fluids Relat Interdiscip Topics 60:1412–1427.

Carlson JM, Doyle J. 2002. Complexity and robustness. Proc Natl Acad Sci USA 99:2538–2545.

Chelikani P, Carpena X, Fita I, Loewen PC. 2003. An electrical potential in the access channel of catalases enhances catalysis. J Biol Chem 278:31290–31296.

Csete ME, Doyle JC. 2002. Reverse engineering of biological complexity. Science 295:1664–1669.

Dasso M. 2001. Running on Ran: Nuclear transport and the mitotic spindle. Cell 104:321–324.

Furuta E, Pai SK, Zhan R, Bandyopadhyay S, Watabe M, Mo Y-Y, Hirota S, Hosobe S, Tsukada T, Miura K, Kamada S, Saito K, Iiizumi M, Liu W, Ericsson J, Watabe K. 2008. Fatty acid synthase gene is up-regulated by hypoxia via activation of Akt and sterol regulatory element binding protein-1. Cancer Res 68:1003–1011.

Gao N, Ding M, Zheng JZ, Zhang Z, Leonard SS, Liu KJ, Shi X, Jiang BH. 2002. Vanadate-induced expression of hypoxia-inducible factor 1 alpha and vascular endothelial growth factor through phosphatidylinositol 3-kinase/Akt pathway and reactive oxygen species. J Biol Chem 277:31963–31971.

Gao N, Shen L, Zhang Z, Leonard SS, He H, Zhang XG, Shi X, Jiang BH. 2004. Arsenite induces HIF-1alpha and VEGF through PI3K, Akt and reactive oxygen species in DU145 human prostate carcinoma cells. Mol Cell Biochem 255:33–45.

Gao P, Zhang H, Dinavahi R, Li F, Xiang Y, Raman V, Bhujwalla ZM, Felsher DW, Cheng L, Pevsner J, Lee LA, Semenza GL, Dang CV. 2007. HIF-dependent antitumorigenic effect of antioxidants in vivo. Cancer Cell 12: 230–238.

Gruss OJ, Carazo-Salas RE, Schatz CA, Guarguaglini G, Kast J, Wilm M, Le Bot N, Vernos I, Karsenti E, Mattaj IW. 2001. Ran induces spindle assembly by reversing the inhibitory effect of importin alpha on TPX2 activity. Cell 104:83–93.

Hambardzumyan D, Becher OJ, Rosenblum MK, Pandolfi PP, Manova-Todorova K, Holland EC. 2008. PI3K pathway regulates survival of cancer stem cells residing in the perivascular niche following radiation in medulloblastoma in vivo. Genes Dev 22:436–448.

Harris AL. 2002. Hypoxia—A key regulatory factor in tumour growth. Nat Rev Cancer 2:38–47.

Harris SR, Schoeffner DJ, Yoshiji H, Thorgeirsson UP. 2002. Tumor growth enhancing effects of vascular endothelial growth factor are associated with increased nitric oxide synthase activity and inhibition of apoptosis in human breast carcinoma xenografts. Cancer Lett 179: 95–101.

Krishnan K, Bruce B, Hewitt S, Thomas D, Khanna C, Helman LJ. 2006. Ezrin mediates growth and survival in Ewing's sarcoma through the AKT/mTOR, but not the MAPK, signaling pathway. Clin Exp Metastasis 23:227–236.

Lee BL, Kim WH, Jung J, Cho SJ, Park J-W, Kim J, Chung H-Y, Chang MS, Nam SY. 2008. A hypoxia-independent up-regulation of hypoxia-inducible factor-1 by AKT contributes to angiogenesis in human gastric cancer. Carcinogenesis 29:44–51.

Liu LZ, Hu XW, Xia C, He J, Zhou Q, Shi X, Fang J, Jiang BH. 2006. Reactive oxygen species regulate epidermal growth factor-induced vascular endothelial growth factor and hypoxia-inducible factor-1alpha expression through activation of AKT and P70S6K1 in human ovarian cancer cells. Free Radic Biol Med 41:1521–1533.

Masson N, Willam C, Maxwell PH, Pugh CW, Ratcliffe PJ. 2001. Independent function of two destruction domains in hypoxia-inducible factor-alpha chains activated by prolyl hydroxylation. EMBO J 20:5197–5206.

Mazure NM, Chen EY, Yeh P, Laderoute KR, Giaccia AJ. 1996. Oncogenic transformation and hypoxia synergistically act to modulate vascular endothelial growth factor expression. Cancer Res 56:3436–3440.

Pennacchietti S, Michieli P, Galluzzo M, Mazzone M, Giordano S, Comoglio PM. 2003. Hypoxia promotes invasive growth by transcriptional activation of the met protooncogene. Cancer Cell 3:347–361.

Percipalle P, Butler PJG, Finch JT, Jans DA, Rhodes D. 1999. Nuclear localization signal recognition causes release of importin-alpha from aggregates in the cytosol. J Mol Biol 292:263–273.

Radisavljevic Z. 2003. Nitric oxide suppression triggers apoptosis through the FKHRL1 (FOXO3a)/ROCK kinase pathway in human breast carcinoma cells. Cancer 97: 1358–1363.

Radisavljevic Z. 2004a. Locus of fragility in robust breast cancer system. J Cell Biochem 92:711–715.

Radisavljevic Z. 2004b. Inactivated tumor suppressor Rb by nitric oxide promotes mitosis in human breast cancer cells. J Cell Biochem 92:1–5.

Radisavljevic Z, Gonzalez-Flecha B. 2003. Signaling through Cdk2, importin-alpha and NuMA is required for H_2O_2-induced mitosis in primary type II pneumocytes. Biochim Biophys Acta Mol Cell Res 1640:163–170.

Radisavljevic Z, Gonzalez-Flecha B. 2004. TOR kinase and Ran are downstream from PI3K/Akt in H_2O_2-induced mitosis. J Cell Biochem 91:1293–1300.

Radisavljevic Z, Avraham H, Avraham S. 2000. Vascular endothelial growth factor up-regulates ICAM-1 expression via the phosphatidylinositol 3 OH-kinase/AKT/Nitric oxide pathway and modulates migration of brain microvascular endothelial cells. J Biol Chem 275:20770–20774.

Scheidenhelm DK, Cresswell J, Haipek CA, Fleming TP, Mercer RW, Gutmann DH. 2005. Akt-dependent cell size regulation by the adhesion molecule on glia occurs independently of phosphatidylinositol 3-kinase and Rheb signaling. Mol Cell Biol 25:3151–3162.

Schmelzle T, Hall MN. 2000. TOR, a central controller of cell growth. Cell 103:253–262.

Semenza GL. 2003. Targeting HIF-1 for cancer therapy. Nat Rev Cancer 3:721–732.

Shi Q, Le X, Abbruzzese JL, Peng Z, Qian C-N, Tang H, Xiong Q, Wang B, Li X-C, Xie K. 2001. Constitutive Sp1 activity is essential for differential constitutive expression of vascular endothelial growth factor in human pancreatic adenocarcinoma. Cancer Res 61:4143–4154.

Smith AE, Slepchenko BM, Schaff JC, Loew LM, Macara IG. 2002. Systems analysis of Ran transport. Science 295:488–491.

Sodhi A, Montaner S, Patel V, Gómez-Román JJ, Li Y, Sausville EA, Sawai ET, Gutkind JS. 2004. Akt plays a central role in sarcoma genesis induced by Kaposi's sarcoma herpesvirus-encoded G protein-coupled receptor. Proc Natl Acad Sci USA 101:4821–4826.

Steeg PS. 2003. Angiogenesis inhibitors: Motivators of metastasis? Nat Med 9:822–823.

Tapon N, Moberg KH, Hariharan IK. 2001. The coupling of cell growth to the cell cycle. Curr Opin Cell Biol 13:731–737.

Tazzari PL, Cappellini A, Grafone T, Mantovani I, Ricci F, Billi AM, Ottaviani E, Conte R, Martinelli G, Martelli AM. 2004. Detection of serine 473 phosphorylated Akt in acute myeloid leukaemia blasts by flow cytometry. Br J Haematol 126:675–681.

Trusolino L, Comoglio PM. 2002. Scater-factor and semaphoring receptors: Cell signaling for invasive growth. Nat Rev Cancer 2:289–300.

Uemura H, Ishiguro H, Ishiguro Y, Hoshino K, Takahashi S, Kubota Y. 2008. Angiotensin II induces oxidative stress in prostate cancer. Mol Cancer Res 6:250–258.

Van Etten RA. 2007. Oncogenic signaling: New insights and controversies from chronic myeloid leukemia. J Exp Med 204:461–465.

Wang GL, Jiang BH, Rue EA, Semenza GL. 1995. Hypoxia-inducible factor 1 is a basic-helix-loop-helix-PAS heterodimer regulated by cellular O_2 tension. Proc Natl Acad Sci USA 92:5510–5514.

Wilde A, Lizarraga SB, Zhang L, Wiese C, Gliksman NR, Walczak CE, Zheng Y. 2001. Ran stimulates spindle assembly by altering microtubule dynamics and the balance of motor activities. Nat Cell Biol 3:221–227.

Zhou T, Carlson JM, Doyle J. 2002. Mutation, specialization, and hypersensitivity in highly optimized tolerance. Proc Natl Acad Sci USA 99:2049–2054.