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Locus of Fragility in Robust Breast Cancer System

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Abstract Functional heterogeneous redundancy of breast cancer makes this tumor to be robust. Signaling mechanisms which control cancer responses are crucial for controlling robustness. Identification of locus of fragility in cancer represents basic mechanism to target robustness. The goal of this prospect is to present locus of fragility in breast cancer robust system, and how disruption of this locus induces failure of robustness. My recent research show, that locus of fragility in breast cancer cells is suppression of nitric oxide (NO). When it was targeted, dynamics of cancer to generate robustness failed that it blocked cancer cell proliferation dependent on the NO/Rb pathway, blocked cell migration and angiogenesis dependent on the VEGF/PI3K/AKT/NO/ICAM-1 pathway, and induced breast cancer cell apoptosis through the NO/ROCK/FOXO3a signaling pathway. This tiny and trivial perturbation in breast cancer cells such as suppression of NO represents locus of fragility (weakness) and new approach for breast cancer chemotherapy. J. Cell. Biochem. 92: 1020–1024, 2004.

Key words: breast cancer; locus of fragility; signaling; proliferation; angiogenesis; apoptosis

ROBUSTNESS AND FRAGILITY OF CANCER

The key properties of cell signal transduction networks are robust [Barkai and Leibler, 1997]. Signaling pathways interact with one another and form complex network, and this complexity arise from their relationship and overlapping functions they control [Weng et al., 1999]. Robustness of various cellular processes is rooted in the dynamic interaction of proteins, DNA, and RNA molecules [Barkai and Leibler, 1997; Bhalla and Iyengar, 1999]. Cancers are very complex and heterogeneous disease with a high level of robustness against a range of therapeutic efforts [Kitano, 2003, 2004]. Principle of robustness an error tolerant or system insensitive to the precise values of the biochemical parameters is characteristic of all cellular networks [Barkai and Leibler, 1997; Jeong et al., 2000]. High optimized tolerance theory (HOT) from statistical physics is conceptual framework to study fundamental aspects of complexity [Carlson and Doyle, 2002]. HOT theory postulated that system which acquires robustness against common perturbation tends to be extremely fragile to some unexpected, tiny (trivial) perturbation [Carlson and Doyle, 1999, 2002; Csete and Doyle, 2002]. HOT system has high performance, high structural internal complexity with high densities of interaction, simple robust external behavior and reliability, with the risk to potentially cascading failure initiated by possibly quite small perturbations [Carlson and Doyle, 2002]. These small perturbations represent locus of fragility in cancer robust system. In this prospect, I am presenting how cancer robust system failed, when locus of fragility was targeted by "trivial" small perturbations. I use HOT theory to present my recent discovery of three overlapping signaling pathways in apoptosis, proliferation, and angiogenesis of breast cancer cells as new approach for breast cancer

Abbreviations used: FOXO3a (FKHRL1), forkhead transcriptional factor; VEGF, vascular endothelial growth factor; ICAM-1, intracellular adhesion molecule-1; NO, nitric oxide; NOS, nitric oxide synthase; Rb, retinoblastoma; PI3K, phosphoinositol-3-OH kinase; Cdk, cyclin-dependent kinase; HIF-1α, hypoxia inducible factor; HOT, high optimized tolerance theory; HGF, hepatocyte growth factor; ARNT, aryl hydrocarbon receptor nuclear translocator, known as HIF-1B.

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chemotherapy and for scientific thinking in complex and robust systems such as cancer.

NITRIC OXIDE (NO) SUPPRESSION AS LOCUS OF FRAGILITY IN CANCER

Human breast carcinoma cells have high NO synthase (NOS) activity [Harris et al., 2002a], and produce great amount of NO [Kampa et al., 2001]. When NO suppression was induced three overlapping signaling pathways were affected [Radisavljevic et al., 2000, 2003, 2004] (Fig. 1). In one pathway apoptosis was induced through the NO/ROCK/FOXO3a (PI3K/Akt/caspase-3 independent) pathway [Radisavljevic, 2003], and simultaneously two other overlapping pathways were suppressed for proliferation dependent on NO/Rb pathway [Radisavljevic, 2004] and for cell migration and angiogenesis dependent on the VEGF/PI3K/AKT/NO/ICAM-1 pathway [Radisavljevic et al., 2000]. NO suppression represents locus of fragility (weakness) for all three overlapping and interactive signaling pathways in breast cancer cells.

NO Suppression Induced Apoptosis

NO suppression induces apoptosis through the NO/ROCK/FOXO3a pathway which is PI3K/Akt/caspase-3 independent pathway in human breast cancer cells. Apoptosis is mediated by transcriptional factor FOXO3a (FKHRL1), a downstream protein from ROCK kinase. NO is negative regulator of ROCK kinase which phosphorylates single thr-32 residue and activates FOXO3a protein [Radisavljevic, 2003] inducing apoptosis in breast cancer cells. NO suppression represents locus of fragility in the NO/ROCK/FOXO3a apoptotic pathway.

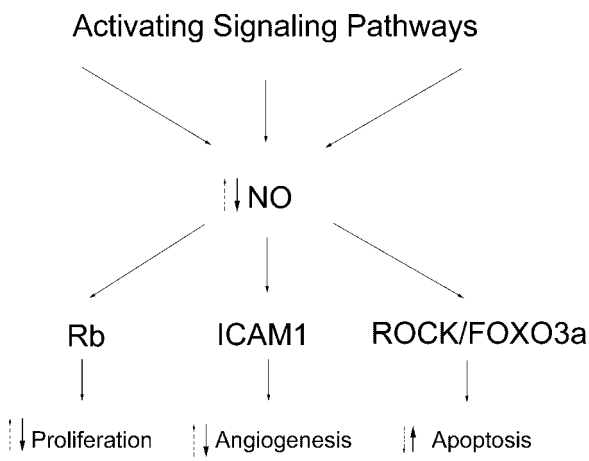

Fig. 1. Locus of fragility in three overlapping signaling pathways. Proliferation controlled via the NO/Rb pathway, angiogenesis controlled through the VEGF/PI3K/AKT/NO/ICAM1 pathway, and apoptosis induced through the NO/ROCK/FOXO3a signaling pathway. Locus of fragility in robust breast cancer system is NO suppression which induced breast cancer cells apoptosis and simultaneously blockage of proliferation and angiogenesis (solid arrow). Activating signals are growth factors such as: hepatocyte growth factor (HGF), vascular endothelial growth factor (VEGF), hypoxia-inducible factor-1α (HIF-1α), L-arginine.

Advanced-stage of human breast invasive carcinoma is correlated with a higher expression of iNOS and high level of NO in cancer cells which contributes to the promotion of apoptosis and angiogenesis in breast carcinoma patients [Mortensen et al., 1999; Reveneau et al., 1999; Vakkala et al., 2000]. Apoptosis in high NO environment is associated with a high concentration of reactive nitrogen metabolites, specifically peroxynitrite [Dimmeler and Zeiher, 1997; Felley-Bosco, 1998]. In NO-mediated apoptosis of breast carcinoma cells [Felley-Bosco, 1998] and other cell-producing NO [Brune et al., 1998] accumulation of tumor suppressor protein p53, caspase activation, chromatin condensation, and DNA fragmentation occurred [Pervin et al., 2001; Jadeski et al., 2002]. Conversely, the physiologic concentration of NO inhibits apoptosis [Ambs et al., 1997] and cell protection is associated with the up-regulation of protective proteins, such as cyclooxygenase-2 or heme-xygenase-1 [Brune et al., 1998]. Activation of serine/threonine kinase ROCK-I as an effector protein during apoptosis, results from caspase-3-mediated activation [Coleman et al., 2001]. Caspase-3 mediated cleavage of ROCK-I induced phosphorylation of the myosin light chain, as well as apoptotic membrane blebbing [Sebbagh et al., 2001]. However, MCF7 breast carcinoma cells lacking the caspase-3 gene were sensitive to apoptosis induced by an unknown mechanism [Janicke et al., 1998], and this mechanism appear to be through the NO/ROCK/FOXO3a pathway which is caspase-3 and PI3K/Akt independent [Radisavljevic, 2003].

Targeting NO Affects Cancer Proliferation

Cell proliferation is induced by growth factors where controlling checkpoints of cell cycle is very important. The retinoblastoma tumor suppressor (Rb) protein regulates proliferation of mammalian cells by maintaining the integrity of the G_1/S checkpoint [Weinberg, 1995]. In majority of human malignancies aberrancies

occur in Rb-pathway [Senderowicz, 2000]. All human tumors have inactivated either the Rb or p53 pathway. The phosphorylation of p53 ser-15 is strongly associated with the response to DNA damage [Kapoor et al., 2000]. Phosphorylated and activated p53 moves to the nucleus, binds to DNA, and coordinates a change in the balance of gene expression leading to growth arrest or apoptosis that prevent the growth or survival of damaged cells [Oren, 1999]. Rb is phosphorylated and inactivated by a family of serine/threonine kinases such as cyclin-dependent kinases (Cdk) [Morgan, 1997]. In the middle-to late G_1 phase to the progression to the S phase of the cell cycle upon mitogenic stimulation, Rb protein is phosphorylated and inactivated [Hatakeyama and Weinberg, 1995]. Loss in Rb function results from the phosphorylation of this protein, however, activation of Rb protein through dephosphorylation arises in cells upon exit from M phase in response to DNA damage [Senderowicz, 2000]. Phosphorylated Rb is unable to bind and inactivate its downstream target the transcriptional factor E2F, leading to transactivation of E2F-dependent genes and progression to S phases [Dyson, 1998].

Breast cancer cells have great production of NO that contributes to the cancer growth and spread [Vakkala et al., 2000] and NO trigger entry into S phase and facilitate mitosis [Plachta et al., 2003]. Recent reports show that tumor suppressor protein Rb was phosphorylated and inactivated by NO without involvement of p53 tumor suppressor protein, leading to increase in breast cancer cells proliferation [Radisavljevic, 2004]. However, when NO was blocked, breast cancer cell proliferation was suppressed [Radisavljevic, 2004]. This observation show, that NO is locus of fragility in the NO/Rb pathway of proliferating breast cancer cells.

Targeting NO Affects Angiogenesis

Proliferating tumors become malignant, when neoplastic cells move and settle into surrounding tissue or distant organs. Main mediators for neoplastic invasive growth are scatter factors, mainly hepatocyte growth factor (HGF) [Nakamura et al., 1989] which acts through the MET tyrosine kinase receptor [Schiering et al., 2003]. Scatter factors also include macrophage-stimulating protein [Skeel et al., 1991], which is recognized by RON, a receptor tyrosine kinase that shares extensive homology with MET [Gaudino et al., 1994]. The extracellular regions of both MET and RON display structural similarities with semaphorins and their receptor plexins also involved in cancer invasion and metastasis [Kolodkin et al., 1993]. In areas distant from new microvessels formatted during angiogenesis in cancer growth and metastasis [Folkman, 1995], hypoxia (a reduction in the normal level of tissue oxygen tension) is present [Harris, 2002b]. In these areas distant from new microvessels, hypoxia is stimulus for production of transcriptional factor hypoxia-inducible factor-1α protein (HIF-1α), which dimerizes with HIF-1β (ARNT) in nucleus to form HIF-1 [Harris, 2002b] which then stimulates production of HGF [Trusolino and Comoglio, 2002], vascular endothelial growth factor (VEGF) [Mazure et al., 1996] and upregulation of MET protooncogene [Pennacchietti et al., 2003], causing increase in invasive growth and metastasis [Harris, 2002b; Trusolino and Comoglio, 2002; Pennacchietti et al., 2003; Semenza, 2003; Kitano, 2004]. VEGF promote angiogenesis through the PI3K/AKT/NO/ICAM-1 pathway and when NO is blocked, angiogenesis was blocked [Radisavljevic et al., 2000]. On the other hand, NO decreases ubiquitination and degradation of HIF-1α, a regulator of metabolic adaptation to hypoxia [Metzen et al., 2003]. Deficit in the HIF-1α decreases ATP and glycine production in cancer [Harris, 2002b]. In normoxia HIF-1α is bound to the tumor suppressor Von Hippel-Lindau protein ubiquitylated and degradated in the proteasome [Metzen et al., 2003]. When angiogenesis induced by VEGF through the PI3K/AKT/NO/ICAM-1 pathway was blocked by NO inhibition [Radisavljevic et al., 2000], simultaneously ubiquitination and degradation of HIF-1α were increased which consequently decreased the VEGF and HGF, resulting in blockage of angiogenesis and cancer invasion abilities. In this angiogenic pathway through the VEGF/PI3K/AKT/NO/ICAM-1, NO is the locus of fragility, because when it is blocked integrity of the pathway was disrupted and angiogenesis was blocked [Radisavljevic et al., 2000].

CONCLUDING REMARKS

Cancers are robust, complex, and heterogeneous disease. Robust or error tolerant system maintains stable functioning, high performance, and internal complexity with the risk to potentially cascading failure initiated by small perturbations if specific parameter is

targeted in the complex signaling pathways. The target represents locus of fragility in cancer robust system. Integrity of pathways generates robustness and redundancy of cancer and disruption of locus of fragility in these pathways abolish cancer robust system. NO suppression phenomenon affects three overlapping and interactive signaling pathways, inducing apoptosis and simultaneously suppressing proliferation and angiogenesis in breast cancer cells. This phenomenon represent locus of fragility in the cancer robust system which opens new approach for breast cancer and other NO producing cancers chemotherapy.

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