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Chromosome Instability and Tumor Lethality Suppression in Carcinogenesis

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ABSTRACT

The maintenance and survival of each organism depends on its genome integrity. Alterations of essential genes, or aberrant chromosome number and structure lead to cell death. Paradoxically, cancer cells, especially in solid tumors, contain somatic gene mutations and are chromosome instability (CIN), suggesting a mechanism that cancer cells have acquired to suppress the lethal mutations and/or CIN. Herein we will discuss a tumor lethality suppression concept based on the studies of yeast genetic interactions and transgenic mice. During the early stages of the multistep process of tumorigenesis, incipient cancer cells probably have adopted genetic and epigenetic alterations to tolerate the lethal mutations of other genes that ensue, and to a larger extent CIN. In turn, CIN mediated massive gain and loss of genes provides a wider buffer for further genetic reshuffling, resulting in cancer cell heterogeneity, drug resistance and evasion of oncogene addiction, thus CIN may be both the effector and inducer of tumorigenesis. Accordingly, interfering with tumor lethality suppression could lead to cancer cell death or growth defects. Further validation of the tumor lethality suppression concept would help to elucidate the role of CIN in tumorigenesis, the relationship between CIN and somatic gene mutations, and would impact the design of anticancer drug development. J. Cell. Biochem. 105: 1327–1341, 2008.

KEY WORDS: SYNTHETIC SURVIVAL; HIGH-COPY SUPPRESSION; TUMOR LETHALITY SUPPRESSION; CHROMOSOME INSTABILITY (CIN); TUMOR LETHALITY SUPPRESSION ADDICTION

Cancer is a disease resulted from genetic and epigenetic alterations that impact on the activities of pathways controlling normal cellular processes [Mendelsohn et al., 2008]. It is now ranked the second death-causing disease in the North America. There are more than 200 cancer types and about 350 cancer genes have been discovered, and more are waiting to be identified [Weinberg, 2007]. These varieties have been challenging several cancer research areas including cancer classification, diagnosis, treatment and prognosis for decades. It has been proposed that cancer may have the following eight common traits: self-sufficiency in cell growth signaling; resistance to inhibitory growth signaling; evasion of apoptosis; unlimited lifespan; sustained angiogenesis; invasion and metastasis [Hanahan and Weinberg, 2000], escape of immune system and genome instability [Weinberg, 2007]. Currently, the most hotly debated trait of cancer is genome instability. The integrity of chromosome number and structure is essential for the survival of an organism and defects of it would result in fatal diseases or birth abolition. In contrast, cancer cells, especially solid tumor cells not only have numerous somatic gene mutations but also are chromosome instability (CIN: gain of extra chromosomes, loss of chromosomes, chromosome breakages, reciprocal and non-reciprocal chromosome translocations, massive DNA fragment insertion or deletion) [Kops et al., 2005]. It has being disputed for a century whether CIN contributes to tumorigenesis or it is just a remnant of oncogenic transformation [Marx, 2002; Rajagopalan and Lengauer, 2004; Torres et al., 2008]. Even though it is well accepted that cancer is triggered by accumulation of several mutations and it has been estimated that most tumors require six to ten genetic changes, there is no agreement on how incipient cancer cells acquire so many mutations and chromosomal abnormalities [Marx, 2002; Rajagopalan and Lengauer, 2004]. Different theories and hypotheses have been proposed to address the mechanism of tumorigenesis. Somatic cell mutation theory, which had dominated the past century, proposes that successive DNA mutations in a single cell lead to cancer [Loeb, 1991; Kinzler and Vogelstein, 1996; Hanahan and Weinberg, 2000]. Mutator phenotypic hypothesis

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holds that the normal mutation rate of DNA polymerases are not sufficient to cause the multiple mutations found in human cancers, and that cancer cells must exhibit a mutator phenotype early in tumorigenesis (the mutation rate in the cancer cells is much higher than that in normal cells). It is further proposed that errors in DNA replication and repair account for the multiple mutations in cancer [Loeb, 1991; Bielas and Loeb, 2005; Loeb et al., 2008]. Aneuploidy theory argues that aneuploidy (a kind of CIN where the chromosome number is not an exact multiple of the usually haploid number) causes cancer [Boveri, 1902] and defects in the mitotic assembly checkpoint generate aneuploidy and might facilitate tumorigenesis [Kops et al., 2005]. Genome instability theory thinks that CIN is an early event during tumorigenesis and might therefore be involved in tumor initiation by enhancing gene mutations [Duesberg et al., 2000; Aguilera and Gomez-Gonzalez, 2008; Geigl et al., 2008]. Cancer stem cells (CSCs) theory states that tumors contain a subset of cells that both self renew and give rise to differentiated tumor cells. CSC has been suggested to maintain the indefinite tumor growth. While the majority of tumor cells are destined to die, the self-renewal properties of the CSCs are thought to be the real driving force behind tumor growth [Reya et al., 2001; Rossi et al., 2008]. The tissue organization field theory states that proliferation is the default state of all cells, and carcinogenesis and neoplasia are defects of tissue architecture [Sonnenschein and Soto, 2008]. Each has contributed to the explanation of tumorigenesis and in combination formed the primary principles for current cancer research.

Therefore, most current cancer research activities, aiming at elucidating the biology of cancer and finding cures for cancer patients, are primarily based on the theories proposed more than two decades ago, such as oncogene, tumor suppressor gene and two-hit hypothesis [Knudson, 1984, 2001; Weinberg, 1984, 1991; Varmus, 2006]. Conceptual revolution in cancer research will break through the bottlenecks in the study of both cancer biology and translational research.

GENOME SURVEILLANCE SYSTEMS PREVENT NORMAL CELLS FROM LETHAL GENETIC ABERRATIONS

To survive during the evolution of 6 billions of years, each existent organism has acquired numerous conserved surveillance systems to cope with all kinds of extracellular and intracellular genotoxins, such as ultraviolet, ionizing radiation (IR) and reactive oxygen species. When there is a perturbation, cell cycle checkpoints are activated and prevents the progression into the next phase, thereby providing time for the error to be fixed and maintaining the integrity of chromosome structure and number [Hartwell and Kastan, 1994; Sherr, 2000, 2004; Zhou and Elledge, 2000; Osborn et al., 2002; Lew and Burke, 2003; Bartek et al., 2004; Kastan and Bartek, 2004; Weaver and Cleveland, 2005; Musacchio and Salmon, 2007]. Single-strand or double-strand DNA breaks lead to the activation of DNA damage checkpoints, which in turn prevent cell cycle progression, keep the stability of DNA replication forks and help the activation and recruitment of repair machine to the damaged DNA sites [Sancar et al., 2004; Harrison and Haber, 2006; Su, 2006; Harper and Elledge, 2007; Branzei and Foiani, 2008]. The most important genome guardians are the potent DNA repair networks including base excision repair (BER) [Memisoglu and Samson, 2000], nucleotide excision repair (NER) [Friedberg, 2001; Costa et al., 2003], homologous recombination (HR) [Sung and Klein, 2006], non-homologous end joining (NHEJ) [van Gent and van der Burg, 2007; Weterings and Chen, 2008], which frequently survey the genome integrity along the chromosomes, recognize and fix errors rapidly during normal cell growth. On the other hand, when cells encounter with a bulk of DNA damages that cannot be repaired instantly, the DNA damage checkpoints are activated to co-ordinate DNA damage repair and cell cycle progression [Rouse and Jackson, 2002; Harrison and Haber, 2006; Branzei and Foiani, 2008]. However, most of the checkpoints are double-edged swords. Depending on the extent of damage, they can either rescue or kill a cell. When the damage is reparable, checkpoints will prevent the cell from dying, otherwise they will activate a signal cascade to promote the demise of the cell with intolerable DNA damages via apoptosis, autophagy or mitotic catastrophe. The convergence of these checkpoints seems to be the tumor suppressor p53. Aberrant cell growth, oncogene activation and DNA damage leads to p53 activation. Once activated, it activates and at the same time represses a series of gene expression to determine the fate of a cell with aberrant genome: apoptosis, senescence or rescuing by repair [Lowe et al., 2004; Harris and Levine, 2005; Rodier et al., 2007; Riley et al., 2008]. Thus, under ever-changing growth environments, hard-wired with these double-edged checkpoints and repair networks, the genome integrity of each organism is secured by sustaining the survival of cells with reparable DNA damages and removing the cells with lethal genetic changes.

DYSEREGULATION OF CELL CYCLE PROGRESSION LEADS TO CIN

One of the most challenging paradoxes in both cell biology and cancer research is the CIN in cancer cells, especially in solid tumors. The agreement is that aneuploidy arises from defects in cell cycle progression [Lengauer et al., 1998; Aguilera and Gomez-Gonzalez, 2008; Geigl et al., 2008].

CIN BY ABERRANT MITOSIS

It is widely believed that aneuploidy results from defects of mitosis regulations. First, aberrant mitotic divisions produce cells that enter the subsequent mitosis with multipolar spindles, leading to aneuploidy. These aberrant mitotic divisions may result from previous cytokinesis defects, cell–cell fusion or endoreduplication, and defects in centrosome duplication, maturation or segregation [Kops et al., 2005]. Second, aneuploidy can arise as a result of chromosome cohesion defects. Right following DNA replication in S-phase, the sister-chromatids are bound together by a ring-like structure called cohesin composed of Smc1, Smc3, Scc1, and Scc3. In mammalian cells, during the progression from S phase to prometaphase, the cohesin around the arms of bound sister chromatids is cleaved, but the cohesin at centromere is kept intact till metaphase to anaphase transition, which is involved in the

anaphase promoting complex (APC^{cdc20}) mediated degradation of securin and separase mediated cleavage of Scc1. Defects in the regulation of cohesion result in failure of chromosome segregation [Nasmyth, 2002; Nasmyth and Haering, 2005]. Third, improper kinetochore-mitotic spindle attachment can lead to aneuploidy. Kinetochore, a supper complex structure composed of more than 100 proteins, is both the target and regulator of mitotic assembly checkpoint. Mutations of the components of kinetochore lead to its defective attachment to mitotic microtubules and hence mitosis failure [Burke and Stukenberg, 2008; Cheeseman and Desai, 2008]. Finally, aneuploidy can occur via defects in mitotic assembly checkpoint, which results in chromosome missegregation during metaphase to anaphase transition. Mitotic assembly checkpoint consists of conserved Mad1, Mad2, Bub1, BubR1, Bub3, and Mps1. Mutation of anyone of them results in aneuploidy both in yeast and mammalian cells. Moreover, increasing studies demonstrate the mutations of these genes in human cancers [Lew and Burke, 2003; Musacchio and Salmon, 2007]. Thus the kinetics of chromosome in mitosis is tightly regulated, defects of which lead to aneuploidy and cell death during metaphase and next G1-phase in mammalian cells through mitotic catastrophe and apoptosis.

CIN FROM DNA DAMAGE CHECKPOINTS DEFECTS

Multiple cell cycle checkpoints converge on the regulation of mitosis. Myc-Cylin D1-Rb pathway can affect mitosis progression by controlling the transcription of the essential component of mitotic assembly checkpoint, Mad2 [Hernando et al., 2004; Sotillo et al., 2007]. Since Rb is the central target of cell size checkpoint, G1/S transition checkpoint and ATM/ATR-Chk1/Chk2-p53 DNA damage checkpoint, defect of these checkpoints would results in alterations of mitosis progression under abnormal growth conditions and genotoxins, and thereafter genome instability [Giacinti and Giordano, 2006; van Deursen, 2007]. It is well known that cells with p53, ATM, Chk1 or p16 mutation display genome instability as demonstrated by aneuploidy [Hartwell and Kastan, 1994; Zhou and Elledge, 2000; Bartek et al., 2004; Kastan and Bartek, 2004; Bartek and Lukas, 2007]. In response to DNA damage, incomplete replicated DNA, unrepaired DNA and other genotoxins, yeast cells will finally arrest at proanaphase and die if the insults persist. Under these conditions, the Tel1/Mec1-Rad53/Chk1 (ATM/ATR-Chk2/Chk1 yeast homologue) signaling pathways are activated to prevent cells from progression through mitosis via several mechanisms: (1) by Chk1 mediated inhibition of separase to inhibit the sister-chromosome segregation; (2) by Rad53 mediated inhibition of APC^{cdc20} to prevent metaphase to anaphase transition; (3) by Rad53-Cdc5 (polo-like kinase yeast homologue) mediated inhibition of mitosis exit network (MEN) to inhibit mitosis exit [Wang et al., 2000; Hu et al., 2001b; Harrison and Haber, 2006; Su, 2006; Liang and Wang, 2007]. Analogously, in mammalian cells, mutations of DNA damage checkpoint genes would indirectly lead to aberrant mitotic division, providing the opportunity for CIN and predisposing cells to tumorigenesis [Sherr, 2000, 2004; Harper and Elledge, 2007]. Indeed, most of the checkpoint genes are tumor suppressor genes and are found mutated in a wide range of cancers. For example, more than 50% of tumors have p53 signaling aberrations.

CIN BY DEREGULATION OF CELL SURVIVAL, GROWTH AND PROLIFERATION PATHWAYS

Oncogene activation (RAS, PI3K, AKT, RAF, MYC, SRC, NF-kB) or tumor suppressor gene inactivation (RB, PTEN, TSC) enhances the signaling pathways that promote cell proliferation, survival and growth [Cantley, 2002; Engelman et al., 2006; Shaw and Cantley, 2006]. The Ras-MAPK, PI3K-Akt, and NF-κB are the most prominent ones and currently the major targets for anti-cancer drug exploitation [Lu et al., 2003; Luo et al., 2003; Hennessy et al., 2005; Manning and Cantley, 2007]. For example, more than 70% of cancer cells have mutations in PI3K-Akt signaling and Ras mutation alone occurs in 30% of tumors [Hennessy et al., 2005; Schubbert et al., 2007]. Great advance has been made in elucidating how deregulation of these pathways leads to increased cell proliferation, prolonged survival and upregulated cell growth. For instance, oncogenic upregulation of PI3K-Akt signaling increases the activity of mTOR which in turn promotes cell growth through enhanced macromolecule synthesis via increased protein synthesis [Jacinto and Hall, 2003; Sabatini, 2006; Shaw and Cantley, 2006; Wullschleger et al., 2006; Guertin and Sabatini, 2007]. PI3K-Akt also promotes cell survival through downregulating proapoptotic factors (Bad, FasL, p53) and at the same time upregulating NF-κB pathway [Shaw and Cantley, 2006; Guertin and Sabatini, 2007]. Increasing evidence indicates that these signaling cascades also play an important role in maintaining genome stability [Shen et al., 2007b]. The direct evidence is that Ras, Akt or P110α transformed cells display CIN [Denko et al., 1994; Giaretti et al., 1995; Saavedra et al., 2000; Sallmyr et al., 2008]. But the underlying mechanism that the deregulations of these signaling pathways ultimately lead to CIN is unclear. Recent data suggest that enhanced activity of PI3K-Akt may catalyze CIN by promoting unscheduled sister-chromosome separation, aberrant anaphase initiation and mitosis exit through mTOR-PP2A.

One of the common downstream targets of PI3K and RAS pathways is the mammalian target of rapamycin (mTOR), a central regulator of cell growth and proliferation. The critical upstream regulator of mTOR is the tuberous sclerosis complex (TSC1/TSC2), which integrates signals from growth factors (*via* Ras-Erk1/2 and PI3K-Akt), energy (*via* LKB1-AMPK), oxygen, nutrition and stress, and negatively regulates mTOR activity [Astrinidis et al., 2003; Wullschleger et al., 2006; Guertin and Sabatini, 2007]. $TSC1^{-/-}$ mouse embryonic fibroblasts (MEFs) have increased DNA content, which can be rescued by pretreatment with mTOR inhibitor rapamycin [Astrinidis et al., 2003]. Downstream of mTOR, protein phosphatase 2A (PP2A) is a conserved downstream target and plays an important role for mTOR mediated intracellular balance of protein phosphorylation and dephosphorylation [Duvel and Broach, 2004; Harwood et al., 2008]. Conserved from yeast to human, Shugoshin functions to protect centromere cohesin by recruiting PP2A to reverse cohesin phosphorylation and hence its stability [Clarke and Orr-Weaver, 2006; Kitajima et al., 2006; Riedel et al., 2006; Tang et al., 2006; Yu and Koshland, 2007]. Recent advance in yeast suggests that TOR signaling also regulates metaphase to anaphase transition and mitosis exit [Queralt et al., 2006]. As phosphorylated NET1 releases CDC14 and results in CDC14 activation and anaphase initiation [Stegmeier and Amon, 2004;

Bosl and Li, 2005; de Gramont and Cohen-Fix, 2005; Sullivan and Morgan, 2007], APC^{cdc20}-activated separase downregulates PP2A, leading to increased phosphorylation of NET1 and mitosis progression. Moreover, separase mediated downregulation of PP2A also increases the phosphorylation status of BFA1, which is required to sustain CDC14 activity during mitosis exit [Stegmeier and Amon, 2004; de Gramont and Cohen-Fix, 2005; Queralt et al., 2006]. In addition, we found that the upstream negative regulator of PP2A, TOR, is required for timely mitosis exit by regulating CDC14 [Shen and Bjornsti, unpublished data]. Therefore it is intriguing to speculate that dysregulated PI3K and/or RAS pathways promotes tumorigensis via TSC-mTOR mediated enhanced and premature cleavage of cohesin, unscheduled segregation of sister chromatids and thereafter genomic instability.

TUMOR LETHALITY SUPPRESSION CONCEPT

Given the inevitable spontaneous gene mutation rate during normal chromosome metabolism (10^{-6} mistakes of a normal cycle of DNA replication), the induced mutations derived from extracellular and intracellular genotoxins, the inherent error-prone of some repair mechanisms and the multiple ways to CIN, cells are always at the crossroad of life and death. However, natural selection has empowered cells with the potent checkpoints and repair machinery to maximize cell survival while minimizing mutations. Cells will die of lethal mutations of essential genes such as those that are essential for the very basic life maintenance as DNA replication and energy metabolism. For example, due to the vital roles of the checkpoints and repair machinery in the maintenance of genome integrity and cell survival, mutations of their components would normally lead to cell death. Indeed, mice die at embryo or perinatal with null mutation of these components such as $BRCA1^{-/-}$ [Ludwig et al., 1997], $ATR^{-/-}$ [Brown and Baltimore, 2000; de Klein et al., 2000], and $XRCC4^{-/-}$ [Barnes et al., 1998; Frank et al., 1998]. Moreover, abnormal number or structure of chromosomes that occur during human embryo development results in embryonic lethality [Angell et al., 1986; Generoso et al., 1989; Forsdyke, 1995; Carrell, 2008]. Therefore, normal cells cannot survive mutations of essential genes and CIN [Kops et al., 2004]. Nevertheless, as mentioned above, cancer cells not only have mutations of essential genes but also are genetically instable [Rajagopalan and Lengauer, 2004]. Thus, cancer cells can survive the lethal mutation of housekeeping genes and tolerate CIN. Based on yeast genetic interactions studies, we propose that cancer cells have acquired the ability to suppress the lethality of essential gene mutations and CIN via precedent mutations of non-essential genes and/or non-lethal mutation of essential genes during the course of multistep tumorigenesis. We called this phenomenon as "tumor lethality suppression," the precedent genetic alteration as "tumor lethality suppression mutation" and the affected gene as "tumor lethality suppressor." We will first briefly discuss the yeast genetic interactions, and then describe the tumor lethality suppression in detail.

LETHALITY SUPPRESSION MECHANISM IN YEAST

In yeast, over-expression or increasing the copy number of a gene can suppress the lethality resulted from mutation or deletion of another gene or a set of genes. This genetic interaction is called high-copy suppression. For example, the human ATR homologue MEC1, or CHK2 homologue RAD53 are the major signal transducers of the cell cycle and DNA damage checkpoints of yeast cells and play pivotal roles in maintaining the genomic integrity in response to genotoxins [Desany et al., 1998; Nyberg et al., 2002; Harrison and Haber, 2006; Su, 2006]. Deletion of either of them results in cell death, however, over-expression of ribonucleotide reductase large subunit RNR1 can suppress the lethality of either MEC1 or RAD53 deletion [Desany et al., 1998]; similarly, over-expression of histone chaperone ASF1 also suppresses the lethality of either MEC1 or RAD53 deletion [Hu et al., 2001a]. Conversely, deletion (not over-expression) of another gene or several genes can suppress the death or growth defects of a gene deletion or mutation. This genetic interaction is called synthetic survival. This technique has been widely used to construct some mutant strains, such as deletion of SML1 to suppress lethality of MEC1 deletion (SML1 MEC1 double deletion strain) or RAD53 deletion (SML1 RAD53 double deletion strain) [Zhao et al., 1998]. Both high-copy suppression and synthetic survival are also referred to as dosage suppression or second mutation suppression. Dosage suppression not only can suppress the lethality or growth defects resulting from mutation of a single gene or several genes but also can even tolerate CIN (CIN suppression) [Dorer et al., 1993; Klein, 2001; Zhang et al., 2006; Au et al., 2008]. For instance, deletion of RecQ helicase SGS1 tolerates CIN and interchromosomal rearrangement [Ajima et al., 2002]. Another kind of genetic interaction is synthetic lethality: single deletion of either of two genes does not affect cell viability, while deletion of both results in cell death [Kroll et al., 1996; Hartman et al., 2001; Measday and Hieter, 2002]. For example, yeast strains with deletion of either the yeast claspin homologue MRC1 or the yeast 53BP1 homologue RAD9 are viable, while deletion of both results in cell death [Tong et al., 2004]. Synthetic lethality has been proposed to be the basis for the current development of molecular targeted cancer therapy through drug screening by RNA interference (RNAi) and chemical libraries [Kaelin, 2005]. These yeast genetic interactions are conserved from yeast to mammalian cells and have been widely and successfully used in epistasis analysis to dissect different signal transduction pathways in a diverse number of model systems (Fig. 1).

TUMOR LETHALITY SUPPRESSION MECHANISM IN CANCER BIOLOGY

In eukaryotes, most genes are non-essential. For example, the budding yeast *Saccharomyces cerevisiae* contains about 6,000 functional genes, but only about 30% of them are essential [Tong et al., 2001, 2004; Boone et al., 2007]. According to the sequencing results of the Human Genome Projects, a human genome has about 30,000 genes. If a human cell has a similar essential to non-essential gene rate, it will be less than 10,000 essential human genes. In other words, loss of one or some of human genes may not endanger cell survival and growth. According to the systems biology and networking theory each protein is a node in the complicated signaling networks, altered activities or function of this protein will probably affect the whole signaling pathway or be buffered by the feedback loops in the networks [Sharom et al., 2004]. Therefore, loss

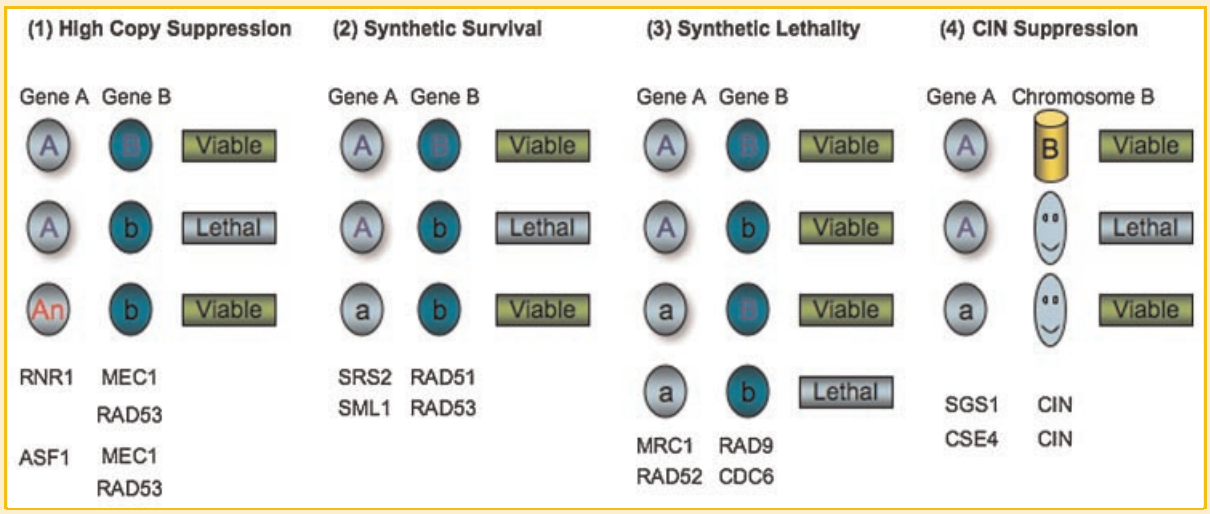

Fig. 1. Yeast genetic interactions. (1) High-copy suppression. Mutation (including gene deletion, over-expression or other loss-of-function mutation) of the essential gene B leads to cell death, while this death can be rescued by simultaneously over-expression of another gene A (*An*). (2) Synthetic survival. If the lethal mutation of gene B can be rescued by deletion of gene A, this genetic interaction is called synthetic survival. (3) Synthetic lethality. Deletion of either gene A or B has no apparent effect on cell viability and growth, but cells will die from simultaneous deletion of both gene A and B. (4) Chromosome instability (CIN) suppression. Loss/gain of any chromosome or fragments of chromosomes (*happy face*) in a cell would lead to cell death or aberrant growth, however this death or growth defects can be rescued by mutation of one or a set of genes. Below each panel shows the samples of individual yeast genetic interaction. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

of one or several non-essential genes will not lead to cell death but probably alter some cell properties such as increased or decreased proliferation rate, sensitivity to environmental stresses and genome stability [Hartman et al., 2001]. For instance, loss of ATM does not affect cell growth under normal conditions but renders cells hypersensitive to IR and predispose cells to tumorigenesis [Shiloh, 2003]. Similarly in yeasts deletion of the large subunit of ribonucleotide reductase RNR3 does not display any phenotypes under normal growth conditions, but is hypersensitive to rapamycin inhibition of the target of rapamycin (TOR) in response to DNA damage [Shen et al., 2007a]; cells with null mutant of histone chaperon ASF1 display increased spontaneous DNA damage but are viable under normal growth conditions [Ramey et al., 2004].

During the multistep process of tumorigenesis, cancer cells might have applied these conserved genetic interactions to acquire the competence to evade the complicated intracellular and extracellular barriers such as immune system, growth inhibitory signals, cell cycle checkpoints and apoptosis [Hanahan and Weinberg, 2000; Hahn and Weinberg, 2002; Vogelstein and Kinzler, 2004], and progress to a specific type of cell, which we called cancer cell. With synthetic survival and/or high-copy suppression, the initial mutations of non-essential genes (p53, for example) may suppress the following lethal mutations of essential genes (MDM4) [Parant et al., 2001] (Table I). These incipient cancer cells with the combined mutations of both non-essential and essential genes might have acquired the ability to further tolerate a larger range and number of gene mutations [Wood et al., 2007], or even gain or loss of fragments or whole chromosomes [Rajagopalan and Lengauer, 2004]. In other words, the preceded accumulation of somatic gene mutations enables the incipient cancer cells to acquire the ability to suppress the lethal essential gene mutation and/or CIN that follows during the multiple-step tumorigenesis [Vogelstein and Kinzler, 1993; Chen et al., 2005]. CIN mediated huge gain and loss of genes provide a even wider buffer for further genes reshuffling and accelerate the evolution and progression of cancer cells, resulting in cancer cell heterogeneity, drug resistance, evasion of oncogene addiction and cancer invasion and metastasis (Fig. 2). For instance, loss-function of p53 mutation suppresses numerous lethal essential gene mutations, predisposes cells to malignant transformation and tolerates CIN during late stage of cancers [Symonds et al., 1994; Jones et al., 1995; de Oca Luna et al., 1996; Connor et al., 1997; Gao et al., 2000; Parant et al., 2001; McAllister et al., 2002; Chen et al., 2005; Hingorani et al., 2005; Siveke and Schmid, 2005; Cao et al., 2006].

Yeast genetic interactions (Fig. 1) provide an explainable theory for the suppression of lethal essential gene mutations by preceding non-essential gene mutations. But how do the non-lethal gene mutations tolerate gain or loss of one or several whole chromosomes? As each human chromosome contains both essential genes

TABLE I. Examples of Tumor Lethality Suppression in Metazoans

Tumor lethality suppressor	Target gene	References
p107	RB	Zhang et al. [2004]
p53	PTEN	Chen et al. [2005]
	RB	Symonds et al. [1994]
	MDM2	Jones et al. [1995]; de Oca Luna et al. [1996]
	MDM4	Parant et al. [2001]
	XRCC4	Gao et al. [2000]
	BRCA1	McAllister et al. [2002]; Cao et al. [2006]
	BRCA2	Connor et al. [1997]; Ludwig et al. [1997]
	K-RAS	Hingorani et al. [2005]
CHK2	BRCA1	Cao et al. [2006]
ATM	BRCA1	Cao et al. [2006]
	XRCC2	Adam et al. [2007]
BCL-2	MYC	Hueber et al. [1997]

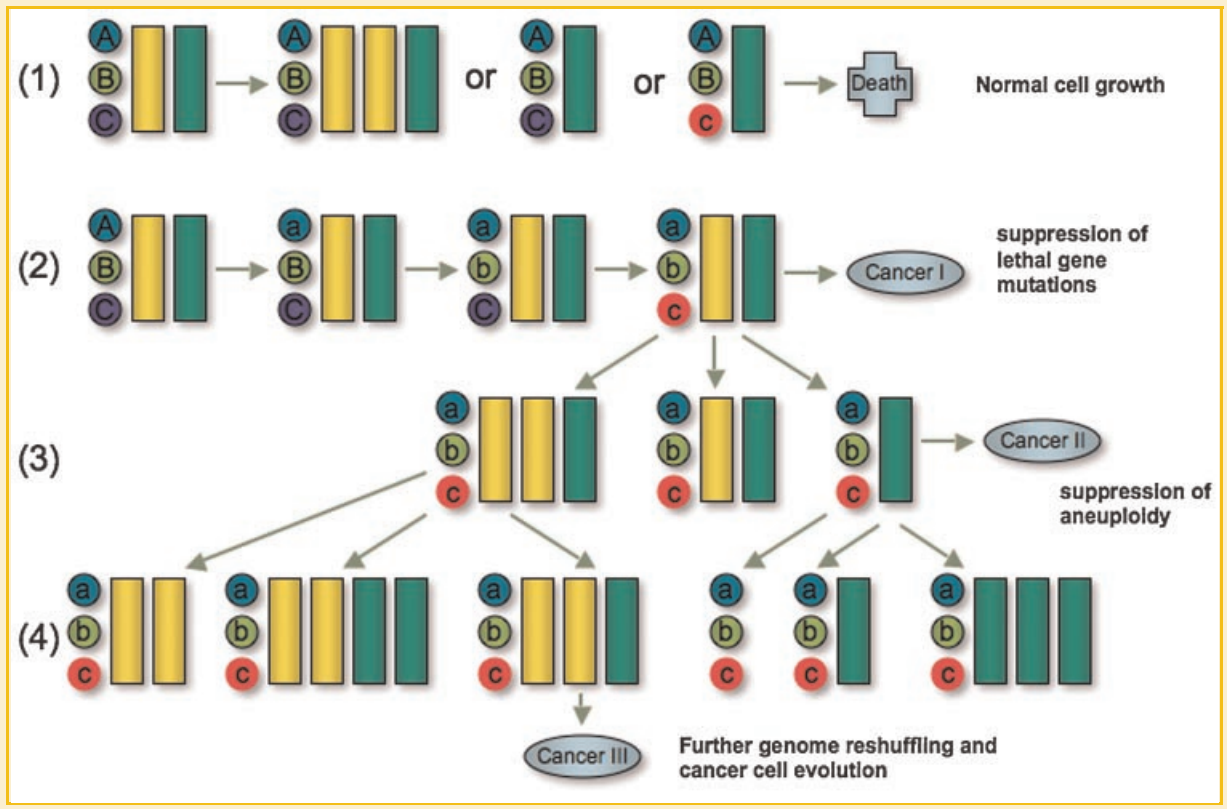

Fig. 2. Tumor lethality suppression in tumorigenesis. (1) Hard-wired with complex genome surveillance systems and potent DNA repair machinery, normal cells and hence organism cannot survive gain/loss of chromosomes or chromosome fragments, or lethal gene mutations under normal cell growth. (2) As gene mutation is inevitable due to the inherent 10^{-6} mutation rate of DNA replication, some error-prone repair machines and transient checkpoint activation, non-lethal mutation of one or several genes (a and b mutations) may suppress the lethal gene mutations (lethal c mutation) (high-copy suppression or synthetic survival). In turn, this lethal mutation may also suppress further lethal mutations. If the lethal mutation happens on a gatekeeper gene, this incipient cell probably is the incipient cancer cell and will develop to a cancer clone. If the lethal mutation happens on a caretaker gene, such as genome maintenance repair or checkpoint gene, this incipient cell becomes genome instable and its gene mutations will be accelerated. Thus, suppression of lethal mutations by preceded non-lethal gene mutations can lead to cancers, such as lymphoma and leukemia. (3) According to yeast chromosome instability (CIN) suppression, when the mutations of non-lethal and lethal mutations accumulate to a certain level, the cell may survive the gain of an extra or loss of a chromosome. Therefore, during the early stages of tumorigenesis, CIN is the effector of somatic gene mutations. (4) During the subsequent divisions of the cancer cells that have obtained both somatic gene mutations and CIN, which is the karyotypes of normal solid tumors, the massive mutations of gain-of-function of oncogene and loss-of-function of tumor suppressor genes during CIN suppression may greatly provide the advantages for the growth, proliferation and survival of the incipient cancer cells and tolerate further large extent of CIN, leading to cancer cell heterogeneity, cancer drug resistance and evasion of initial oncogene addiction. Thus, during the late stage of cancer progression, CIN is the cancer inducer as well as effector. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

and non-essential genes, loss or gain of anyone of the 46 chromosomes would lead to cell death, fatal diseases or birth abolition. However, most human genes are non-essential, loss one or several of them may not affect cell viability. Therefore, in analogue to yeast genetic interactions, it is possible that, during the evolution of cancer cells, when the accumulation of non-essential and non-lethal essential gene mutations reaches certain levels in the incipient cells, it may suppress the lethal loss of the essential genes that are located in a chromosome (CIN suppression mutation). Chromosome loss or gain can happen during the very rare occasion of chromosome missegregation resulted from mitosis defects, checkpoint failure, stress or even normal cell growth [Kops et al., 2005]. In other words, the derived cells now survive the loss of this chromosome. Even though the massive gain or loss of both the essential and non-essential genes in the first lost chromosome does not lead to cell death, it must have perturbed the balance of the molecular circuit of the cellular signaling networks, and made the incipient cell's genome more instable [Torres et al., 2008], which in turn provides a wider buffer to further suppress lethal mutations of essential genes on the other chromosomes and the loss and/or gain of chromosomes in the subsequent cell cycles. After a certain number of cycles of this gene reshuffling, the incipient cells mature to cancer cells with a veritable gallery of horrors: activated or inactivated genes, extra or missing chromosomes, and a host of other genetic abnormalities [Marx, 2002]. It has been estimated that common tumors have 60–90 chromosomes instead of 46 [Rajagopalan and Lengauer, 2004] (Fig. 2).

The premise of our tumor lethality suppression hypothesis is the precedent somatic gene mutations for cells to obtain enough ability to tolerate lethal essential gene mutations and to buffer CIN.

Accordingly, CIN is a late event and accelerates gene reshuffling during tumorigenesis. Thus, CIN is both the effector and inducer of carcinogenesis.

There are numerous examples to support this hypothesis, to name but a few (Table I). The direct and solid evidence is the recent finding that the average breast or colon tumor contains 93 mutated genes, most of which are non-essential [Wood et al., 2007]. Second, transforming mouse cells with mutant oncogenes lead to activation of other oncogenes. For example, Ras transformed cells display Akt activation and depends on the cooperation of these two activated oncogene for tumorigenesis [Li et al., 2004]. Third, loss-of-function of tumor suppressor gene or activation of oncogenes depends on other gene mutations [Zhang et al., 2004; Cao et al., 2006; Adam et al., 2007]. As mentioned above, loss-of-function of p53 mutation suppresses numerous lethal essential gene mutations [Symonds et al., 1994; Jones et al., 1995; de Oca Luna et al., 1996; Connor et al., 1997; Gao et al., 2000; Parant et al., 2001; McAllister et al., 2002; Chen et al., 2005; Hingorani et al., 2005; Siveke and Schmid, 2005; Cao et al., 2006; Adam et al., 2007]. Moreover, Myc induced apoptosis depends on bcl-2 and IGF-1 signaling [Hueber et al., 1997].

MECHANISTIC EXPLANATION BY TUMOR LETHALITY SUPPRESSION CONCEPT IN CANCER

More than three decades have passed by since President Nixon launched the ''War against Cancer'' in 1971, but the underlying mechanism of tumorigenesis is still unclear. Rapid advances in modern molecular biology and biotechnology, the availability of the complete sequence of human genome and the success of targeted therapy, have led people to predict that cancer will be conquered very soon. However, basic cancer research currently seems only to have some minor impact on the control of this disease in clinical practice. Conventional surgery, radiotherapy and cytotoxic chemotherapy, which were developed about a half-century ago, still predominates the clinical applications. Therefore, Varmus [2006] advocated for a deeper understanding of cancer biology to achieve the goal of ''The War against Cancer.'' There are numerous urgent questions that are waiting to be answered. Such as, how do cancer cells become and survive CIN? How do cancer cells accomplish heterogeneity during cancer progression? Why is only a small part of patients with the same tumor type sensitive to a targeted therapy? Why does cancer drug resistance occur so rapidly and widely? ''Oncogene addiction'' can explain some traits of cancer and provide the basis for targeted therapy [Weinstein, 2002; Sharma and Settleman, 2007; Letai, 2008; Weinstein and Joe, 2008], but why do a large part of cancer cells evade ''oncogene addiction'' during molecularly targeted therapies?

Even though we are excited with great potential to develop rational, hypothesis-driven, mechanism-based molecular therapeutics for cancer [Sawyers, 2004; Kaelin, 2005; Collins and Workman, 2006], how the complex signal transduction networks are hijacked by malignant cells is still poorly understood. More than 90% of cancer patients die from the metastasis of cancer cells but the genes responsible for metastasis and the underlying mechanisms are most unclear [Fidler, 2003]. Current studies may have just scratched the iceberg. Tumorigenesis is not driven by the error of a simple linear and textbook pathway, but the perturbation of the important networks that control normal cell growth, proliferation and survival [Vogelstein and Kinzler, 1993, 2004; Hanahan and Weinberg, 2000; Wood et al., 2007; Sawyers, 2008]. To better understand the cancer biology and to develop potent cancer therapeutics, it is necessary to elucidate the feedback and feedforward loops, and the network robustness and sensitivity [Varmus, 2006].

Temporally, both academic and industry are focusing on the exploitation of the drugs directly targeting oncogenes, especially kinome [Collins and Workman, 2006]. Tumor suppressor genes theoretically should be the most efficient and promising targets for cancer therapy, but how to target them? The bottleneck of cancer therapy does not lie in our inability to find chemical compounds to kill cancer cells, but lie in our inability to find the chemicals that can kill cancer cells but spare normal cells [Kaelin, 2005]. In the following sections, we attempt to provide explanation for some of the long-standing questions in cancer research and propose a method for anticancer drug development based on tumor lethality suppression hypothesis.

CANCER HETEROGENEITY

One of the most formidable problems in cancer research is the cancer heterogeneity [Stingl and Caldas, 2007]. Cells from different patients, the same type of cancer cells from different organs, or even cells from the same site of the same patient are heterogeneous, with a subgroup of or even individual cells displaying distinct karyotypes. This cancer heterogeneity places a hurdle for cancer research in almost all aspects [Varmus, 2006]. Because of the difficulty to get homogenous cancer specimens, basic cancer researcher always get varied results with genomic profiling and proteomic techniques such as gene chip, tissue array or protein array to elucidate the molecular events of cancer. Perhaps, the most direct and critical impact of cancer heterogeneity is the insensitivity to targeted cancer therapy, because the cancer cells from even the same patient contain different molecular circuits [Burgess and Sawyers, 2006; Nahta et al., 2006]. The applied drug may only inhibit or kill a subset of cancer cells that depends on that target or signal pathway. The others will be insensitive to it because their survival depends on other targets or signal pathways. How do the incipient cancer cells become heterogeneous? CSC theory holds that cancer cell heterogeneity is due to the different differentiation direction of the CSC under changing microenvironment [Reya et al., 2001; Stingl and Caldas, 2007; Rossi et al., 2008]. According to the tumor lethality suppression theory, cancer cell heterogeneity may be derived from the continuous gene reshuffling after each cycle of CIN tolerance during tumorigenesis. Since gene mutation is inevitable and can happen on every gene in a cell, any single or some combinations of several non-lethal gene mutations will not affect cell viability, but occasionally it may suppress the lethal mutation of some essential genes. If it happens on the components of the genome surveillance systems and DNA repair machinery the genome of the cell will become highly instable and the mutation rate will be accelerated (mutator phenotype) [Loeb, 1991; Bielas and Loeb, 2005; Loeb et al., 2008]. When gene mutations are accumulated enough to tolerate the gain or loss of the first chromosome, the incipient cell

has now become aneuploidy. The massive gain and/or loss of genes accompanied with the gain and/or loss of chromosomes render the incipient cells to obtain the ability to suppress higher grade of CIN, that is, to tolerate more chromosome gain and loss, and results in defects or further defects of mitosis progression. In the subsequent cell cycles, when an aberrant mitosis happens, any daughter cell with gain of chromosomes would be accompanied with another daughter cell with loss of chromosomes. The probability of aberrant mitoses is very high because of the genome instability of the incipient cells. If both daughter cells are viable due to the higher lethality suppression, two populations of cells with different karyotypes now come into being. As cancer cells grow and proliferate very fast, similar split into subsequent two populations of cells are still going on with higher and higher rate. Finally, the mature cancer cells are composed of a mixture of cells with distinct karyotypes (Fig. 2).

ONCOGENE ADDICTION, TUMOR SUPPRESSOR HYPERSENSITIVITY AND EVASION OF ONCOGENE ADDICTION

"Oncogene addiction" holds that cancer cells are addicted to or dependent on one or a few mutated genes (oncogenes and/or tumor suppressor genes) for both maintenance of the malignant phenotype and cell survival. Thus reversal of only one or a few of these abnormalities may induce cancer cell death and/or inhibit cancer cell growth [Weinstein, 2002; Weinstein and Joe, 2008]. Both experimental and clinical evidence supports the concept of "oncogene addiction" [Sharma and Settleman, 2007; Letai, 2008]. For example, conditional transgenic mice expressing an inducible H-*ras* oncogene readily developed melanomas; when expression of H-ras was shut off the melanoma rapidly underwent apoptosis and regressed [Chin et al., 1999]. Success in targeted cancer therapy is perhaps the most solid evidence of "oncogene addiction," such as treatment of chronic myeloid leukemia (CML) patients with imatinib to block mutated Bcr-Abl [Druker, 2004]; curing of non-small cell lung cancer (NSCLC) patients with Erlotinib to inhibit EGFR [Tsao et al., 2005]. Similarly, reintroducing a wild-type tumor suppressor gene into cancer cells with mutation of it can cause marked inhibition of tumor growth, induction of apoptosis and/or inhibition of tumorigenesis in mice. In parallel to "oncogene addiction," this phenomenon was also called "tumor suppressor hypersensitivity" [Weinstein and Joe, 2008]. According to the tumor lethality suppression hypothesis, the survival of cancer cells may depend on the preceded tumor lethality suppressor mutation (in the early stage of tumorigenesis, it is mutated non-essential gene, but at later stage it may also be mutated essential gene) to tolerate essential gene mutations or CIN (tumor lethality suppression addiction), reversal of the mutated tumor lethality suppression would result in cell death or growth defects. If the mutated tumor lethality suppressor is a gain-of-function of oncogene, its inhibition may lead to defective cell growth or death; while if the suppressor is a loss-of-function of tumor suppressor or genome maintenance gene, reversal of it may also meet the goal of cancer therapy.

Although mouse models and clinic application of molecular targeted therapy provide strong evidence for the concept of "oncogene addiction," the same experiences also demonstrate "evasion of oncogene addiction" [Savona and Talpaz, 2008; Weinstein and Joe, 2008]. For example, in c-myc breast cancer model, when c-myc was switched off, only 50% of the breast tumors regressed [D'Cruz et al., 2001]. It was proposed that this is probably due to secondary addiction to another oncogene or to the growth of a population of "non-addicted" cancer cells [Hulit et al., 2001; Weinstein and Joe, 2008]. Savona and Talpaz [2008] suggested that the resistance of CML to imatinib might be due to the primitive imatinib-refractory CML stem cells. According to "tumor lethality suppression" theory, "evasion of oncogene addiction" may be normal, especially in solid tumors with CIN. As stated above, late-stage cancer actually is a mixture of different populations of cell types, with each population dependent on different tumor lethality suppression mutations. The earlier the stage is, the more cancer cells will be dependent on that trigger tumor lethality suppressor mutation for transformation. During the progression of cancer, cells become more and more heterogeneous because of enhanced tolerance of increased CIN rate, different population of cells may have obtained distinct "bizarre circuit" and the original dependence may be switched to other tumor lethality suppressor mutations.

TUMOR LETHALITY SUPPRESSION CONCEPT IN CANCER DRUG DEVELOPMENT

Genetic interactions have emerged as a powerful tool for dissecting the genetic changes of the multistep process of tumorigenesis and providing the theory for targeted cancer therapy [Hartman et al., 2001]. Synthetic lethality is the basis for oncogene addiction phenomenon of cancer cells and provides the theory for the current post-genome era cancer drug screening by RNAi library and chemical library [Kaelin, 2005; Collins and Workman, 2006]. A gene is called a "tumor lethality suppressor," when genetic or epigenetic alterations of it suppress the lethality or growth defects of another gene mutation or CIN. Thus reversal of tumor lethality suppression mutation may lead to cancer cell death or sick. In the following part, we will propose a new method, based on "tumor lethality suppressor," for cancer drug target and biomarker identification and validation.

TARGETING ESSENTIAL GENOME GUARDIAN GENES OR LETHAL ONCOGENE MUTATION VIA MODULATING ITS LETHALITY SUPPRESSOR

Temporally, kinome is the focus for cancer drug development. Many important pathways, such as Ras, Myc and metastasis, have not yet been well drugged. Moreover, targeting phosphatases and protein–protein interactions have proven technically intractable so far. Most importantly, regarding the vital roles in tumorigenesis and wide mutation in cancers, genome guardian genes theoretically should be the most efficient and promising targets for cancer therapy [Varmus, 2006].

Most, if not all, genome integrity guardians work in the cell cycle checkpoints, DNA damage checkpoint, chromosome integrity checkpoints and DNA damage repair networks [Weinberg, 2007]. Furthermore, most cancer cells harbor one or several loss-of-function of genome guardian genes. Mutations in p53 or Rb pathways, for example, are involved in most cancers [Harris and Levine, 2005; Giacinti and Giordano, 2006]; PTEN is mutated in 30–40% solid tumors [Chow and Baker, 2006; Salmena et al., 2008].

Importantly, in contrast to oncogene, which drives tumorigenesis or maintains cancer cell survival by dominant mutations (gain-of-function), it is the recessive mutation (loss-of-function) of genome guardian gene that drives tumorigenesis or maintains cancer cell viability. It is now widely accepted that oncogenes are also required for normal cell growth, proliferation and survival [Weinberg, 2007; Mendelsohn et al., 2008]. Therefore, direct targeting oncogene by small molecular inhibitor, antibody or RNAi will also affect its functions for normal cell growth, proliferation and survival. This may be one of the main reasons why current anticancer drugs demonstrate both low therapeutic index and narrow therapeutic window [Collins and Workman, 2006]. However, directly targeting oncogenes is the current wave of molecular cancer therapeutic strategy, with both academic and industry hectic in drugging oncogene kinome. In sharp contrast, normal cells but not cancer cells have the wildtype genome guardian gene in sporadic cancers. Thus targeting mutated genome guardian gene will exclusively kill cancer cells while spare normal cells. Actually the idea to target mutated genome guardian gene is not new as several labs had ever tried to reintroduce wild type tumor suppressor gene into cancer cells by means of viral vectors, or to correct the mutated tumor suppressor gene via HR [Ternovoi et al., 2006; Pappas et al., 2007]. All of these methods need the transfection of genes or DNA fragment into cancer cells. Due to the safety, availability, cell permeability, stability, intracellular distribution and compartmentalization of gene therapy drugs, cancer gene therapy by reversing mutated tumor suppressor gene is currently very challenging and formidable. Recently, nutlin was found to effectively interfere with Mdm2-p53 interactions, indicating promising application of this kind drug for anti-cancer drug development [Graat et al., 2007; Wang and El-Deiry, 2008]. Thus, drugging the very promising genome guardian gene mutations, especially mutated tumor suppressor genes, is not popular yet, probably due to unavailable techniques.

With the rapid advance in molecular biology and availability of research resources such as the entire human genome sequence, RNAi library, chemical library, microarrays and gene expression library, it is now the time to utilize ''tumor lethality suppression'' concept to elucidate the alterations of molecular circuitry of cancer cells and explore those undrugged, missed, avoided and formidable targets for cancer drug development. There are numerous tumor suppressor genes and genome maintenance genes found in human cancers, such as ATR, Chk1, BRCA1, BRAC2, PTEN and Rb, which play vital roles for cell survival and proliferation and embryo development, therefore we can use tumor lethality suppression phenomenon to target them indirectly for cancer therapy. The following is one of the proposed procedures (see Fig. 3).

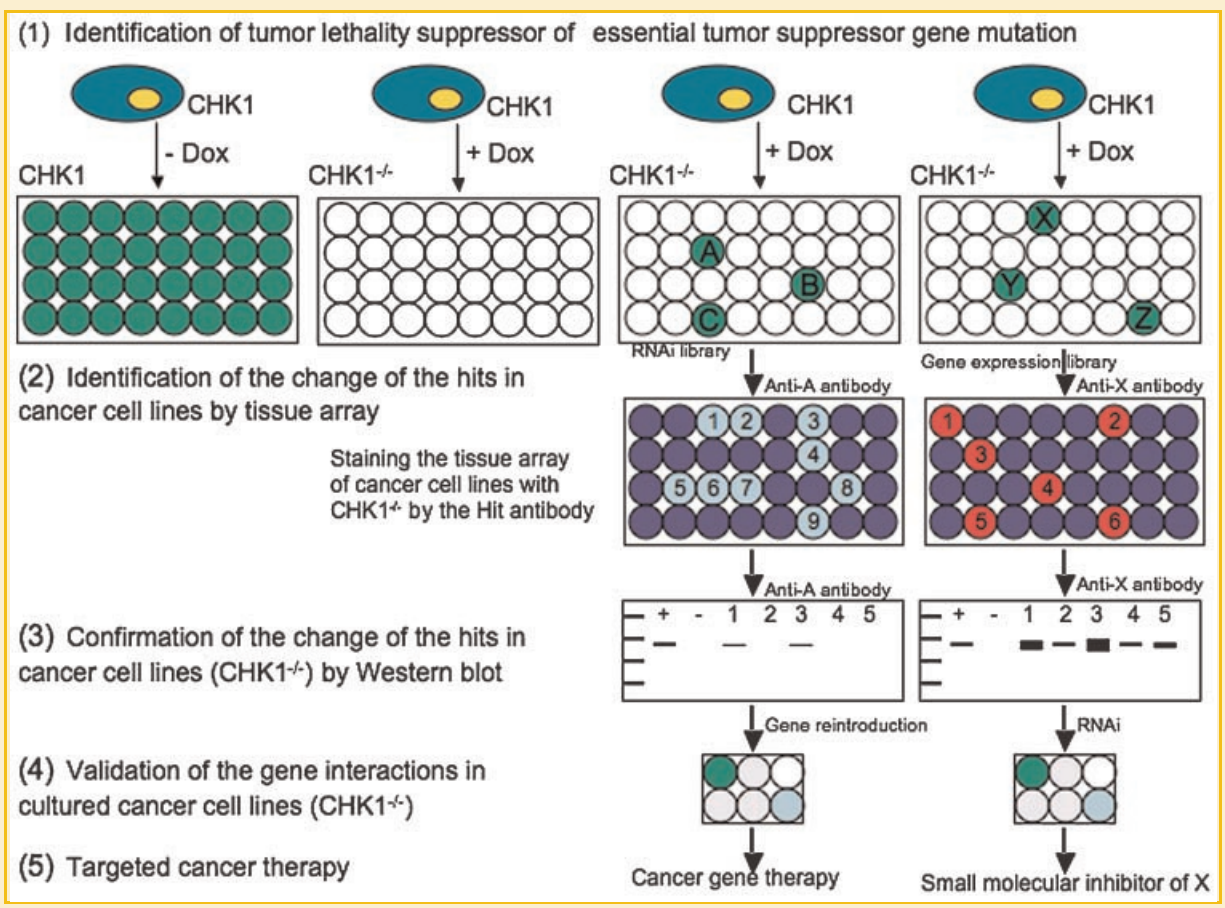

Fig. 3. Targeting the suppressor of tumor suppressor gene mutation for cancer therapy (see text for detail). [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

Identification of lethality suppressor of essential tumor suppressor gene mutation. A cell line containing lethal tumor suppressor gene mutation can be generated by conditional gene-knockout techniques such as Tet-on and Tet-off system. For example, CHK1 deletion will lead to normal cell death or growth defects. However, if the CHK1 gene is under control of Tet-off system, the expression of CHK1 can be turned off by addition of doxycycline (Dox) to the culture medium and results in cell death or growth defects. However this lethality may be rescued by manipulating the suppressor of the lethal CHK1 deletion. First, according to synthetic survival, knockdown of certain gene by RNAi may suppress the lethality or sick of CHK1 depletion. Cells are plated in high-density well plates like 384-well plates with medium without Dox. Individual genes in the whole human genome can be knocked down by transfecting the corresponding RNAi structure (siRNA or shRNA expressing vector) from human RNAi library into each well of cells. After defined time to knock down genes by RNAi, the Dox then is added to the medium to shut down CHK1 expression. Theoretically, cells in most wells will display sick phenotypes due to CHK1 depletion, but in rare cases, knockdown of one gene (for an instance, gene A, B, or C as shown in the figure) may suppress the sick phenotypes from CHK1 deletion, therefore gene A, B, or C are the lethality suppressor of CHK1 deletion. Then we can further validate these suppressors as targets for cancer gene therapy or biomarker for cancer diagnosis, treatment and so on.

On the other hand, based on high-copy suppression, over-expression of certain gene by gene expression library may also suppress the growth alterations of cells with CHK1 deletion. Similarly to the above, individual genes in the whole human genome can be over-expressed by transfecting the corresponding gene expression vectors from human gene expression library into each well of cells. After certain period of time, the Dox is added to the medium to shut off CHK1 expression. Again, cells in most wells will display sick phenotypes due to CHK1 depletion, but in some cases, over-expression of one gene (e.g., gene X, Y, or Z as shown in the figure) may suppress the defects of cell growth resulted from CHK1 deletion, therefore gene X, Y, or Z are also the lethality suppressor of CHK1 mutation. We can further validate these suppressors as targets for targeted molecular cancer therapy by small molecule inhibitor.

Confirmation of protein levels of hits in cancers. After successfully screening the potential suppressors of the lethal cancer gene mutation (CHK1), the protein levels of these potential suppressors will be checked in cancers with known gene mutation (CHK1 in this case). Cancer cell lines or tumor cells with known CHK1 mutation from different patients are cultured in high-density

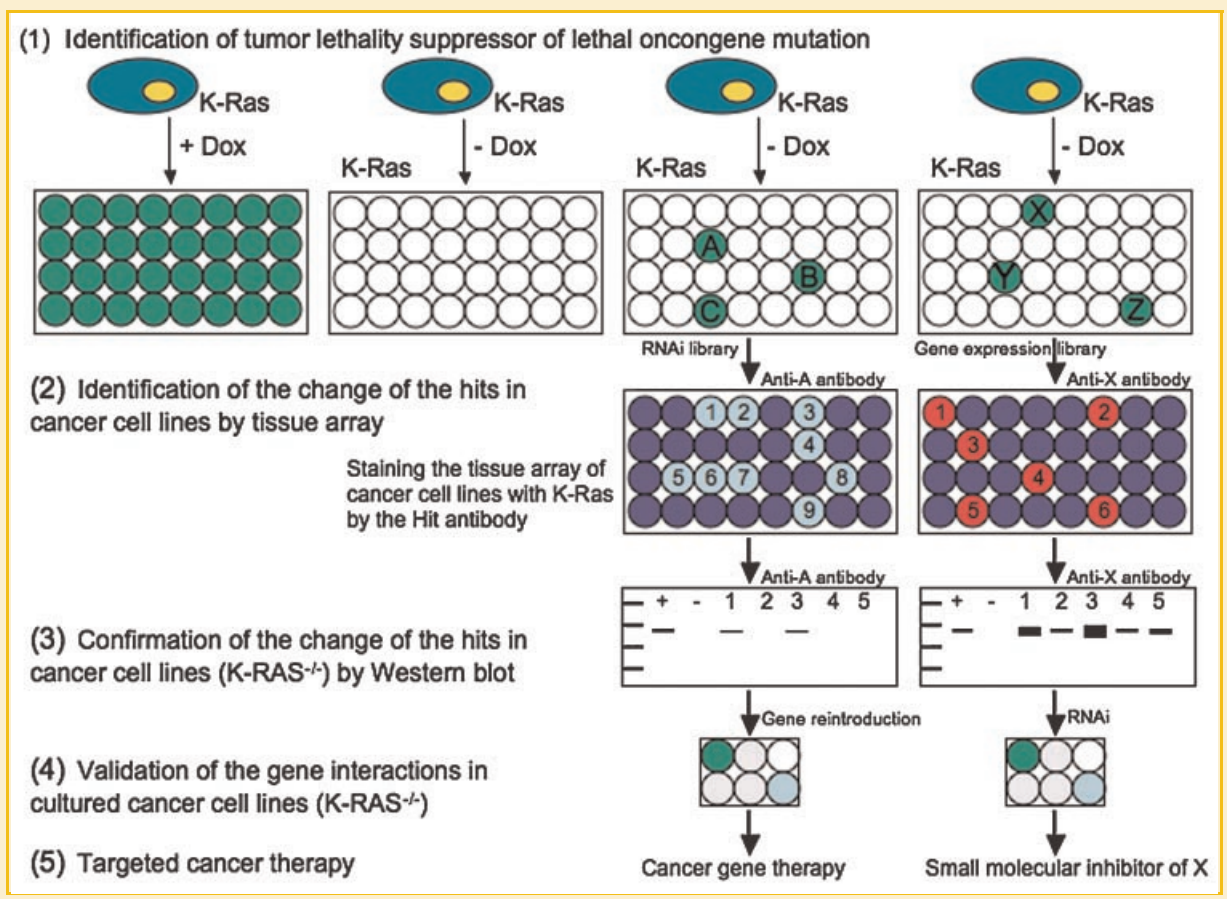

Fig. 4. Targeting the suppressor of oncogene mutation for cancer therapy. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

plates or spotted onto tissue array. The expression level of individual suppressor of CHK1 mutation can be detected by immunohistochemistry or immunocytochemistry with the anti-suppressor antibody (such as anti-A or anti-X antibodies as shown in the figure).

The protein levels of these suppressors that indeed display downregulation or upregulation in the above checked cancers can now be further confirmed by immunoblotting with the respective antibody.

Validation of the gene interactions in cultured cancer cells. After confirming the changes of the protein levels of the potential lethality suppressor of a cancer gene, we can validate them through reversing the gene expression levels by gene reintroduction of the downregulated suppressors or by RNAi for the upregulated ones. In the case of candidate A, if reintroduction of it by transfecting plasmid expressing wild-type A can inhibit cancer cell growth or induced cell death, A therefore can be developed as a target for cancer gene therapy. On the contrary, in the case of candidate X, if downregulation of it by RNAi leads to cancer cell death or growth inhibition, gene X can be also validated as target for targeted molecular cancer therapy by small molecule inhibitors.

The same procedure can be modified for the identification and validation of the lethality suppressor for lethal oncogene mutation, such as K-Ras, for the purpose of cancer therapy and biomarker discovery (see Fig. 4).

CONCLUSION

To evade the lethal barriers imposed by intracellular and host environments, cancer cell has reorganized its intracellular signal circuitry by genetic and epigenetic alterations [Hahn and Weinberg, 2002; Weinberg, 2007]. The mechanism for cancer cells to acquire the enormous genetic and epigenetic alterations and to survive loss of essential cellular processes is still unclear. Why can cancer cells but not normal cells survive lethal gene mutations and/or CIN? Cancer cells must have acquired the capabilities of suppressing the lethal mutations and/or CIN (tumor lethality suppression). In yeast, mutation of one gene or several non-essential genes can suppress the death of a lethal gene mutation or CIN. Similarly, experimental transgenic mice demonstrate that deletion or over-expression of a gene can rescue the death of another lethal gene mutation. During the early stages of the multistep process of tumorigenesis, incipient cancer cells probably have acquired genetic and epigenetic changes that suppress the lethal mutations of other genes and CIN (tumor lethality suppression mutation). Accordingly, compared to normal cell, cancer cell is a disabled one and relies on the preceded genetic and epigenetic changes for growth and proliferation (tumor lethality suppression addiction). Thus, interfering with tumor lethality suppression could lead to cancer cell death or growth defects, suggesting a new avenue for anticancer drug development. The role of CIN in tumorigenesis has been debated for nearly a century. Though it is agreed that all tumors contain small epigenetic and genetic changes in oncogenes and tumor suppressor genes, as well as cytogenetically visible alterations such as chromosome losses, gains and translocations [Cahill et al., 1999], the dispute is still going on about the relationship between somatic gene mutation and CIN, and the functions of CIN in tumorigenesis [Marx, 2002]. According to the tumor lethality suppression, somatic gene mutation is the prerequisite for cell to survive CIN and CIN in turn accelerates tumorigenesis by producing massive mutations. Thus, CIN might be both the effector and inducer of gene mutations mediated birth of cancer.

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