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Operation 'p53 Hunt' to combat cancer: Theaflavins in action

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1. ABSTRACT

With phytochemicals executing a plethora of anti-tumor mechanisms, targeting the 'guardian angel' p53 appears to be a critical strategy to energize the process of cancer therapeutics. Regulation of anti-tumor p53 functions by dietary plant polyphenols particularly black tea and its active component theaflavins has gained immense recognition from the point of view of both efficacy and safety. This review highlights the complexities of p53 functions, molecular mechanisms of its inactivation in cancer, and therapeutic strategies for rescuing p53 dysfunction in tumors using theaflavins. It describes how theaflavins, by steering a single molecular target - p53, regulate multiple hallmarks of carcinogenesis i.e., tumor glycolysis, angiogenesis, metastasis, apoptosis and drug resistance. Additionally, considering the rising of the current concept of cancer stem cells (CSCs), the sole participant in tumor evolution, the review discusses about the possible role of theaflavin-p53 cross talk in targeting CSCs. Such attempts to target the complexities of p53 functions during neogenesis will be of immense help in developing a "new" strategy for successful cancer prevention and therapy by theaflavins.

2. INTRODUCTION

Cancer cells are known to have alterations in multiple cellular signaling pathways and because of these complexities in the communication between multiple signaling networks, the treatment and the cure for most human malignancies is still an open question. The past decades have witnessed chemo/radiotherapy as the mainstay of systemic therapy for both solid and hematological malignancies; nevertheless these modalities have suffered from drawbacks like systemic toxicity and immunosuppression (1). To resolve such downsides, the cancer therapy modalities need to be advanced with more effective and tolerable treatments to specifically target the malignant cell with minimal adverse consequences. Although there is no 'magic bullet' that can completely conquer cancer, more than 250 population based studies, including case control and cohort studies, indicate that people who consume about five servings of fruit and vegetables a day have approximately half the risk of developing cancer (2). Wide arrays of phenolic substances, those present in dietary and medicinal plants, have been reported to possess substantial anti-carcinogenic and anti-

mutagenic effects (3). In addition cancer-induced immunosuppression has been ameliorated by a wide array of phytochemicals (4-11). It is important to note that though each of these phytochemicals is a potent inhibitor of cancer development, they are also non-toxic to the normal cells. What really sets apart their differential effects in abnormal cancer cells verses normal cells is their ability to induce apoptotic pathways in cancer cells while at the same time they protect normal cells by manipulating levels of metabolic and detoxifying enzymes (12). Because of their safety and the fact that they are not perceived as medicine, anticancer phytochemicals have high potential for development as chemopreventive and therapeutic agents that may find widespread and long-term use.

Remarkable advances in the cellular and molecular genetics of carcinogenesis such as the identification of numerous oncogenes, tumor suppressor genes, specific genes encoding carcinogen-metabolizing enzyme, DNA-repair proteins, and regulators of cell cycle and apoptosis have given us a better insight into the process of neoplastic transformation. Moreover *in-vitro* studies are now being conducted to identify the molecular targets/signaling pathways within cancer cells that are modulated by dietary constituents (13). The major molecular targets in cancer therapy include oncogene products, growth factors and their receptors, signal-transducing molecules, hormone receptors, cell cycle-related proteins, telomerase-related molecules, apoptosis-related molecules, angiogenesis-related molecules, anticancer drug resistance/sensitivity factors, transcription factors, and molecules related to infiltration and metastasis. Despite this progress, the identification of molecular and cellular targets of chemopreventive phytochemicals is still incomplete. Many of the molecular alterations that are associated with carcinogenesis occur in cell-signaling pathways at the cross roads of cell survival and apoptosis. One of the central components of the intracellular signaling network that rescues us by dictating the death warrant to tumor cells is our 'guardian angel'- p53. If so, p53 would be expected to play an important role in cancer treatment, with its loss or mutation predicting a substantially worsened prognosis. In fact, loss of p53 oncoprotein appears to decrease the susceptibility towards apoptosis suggesting that defying death may be a fundamental component of neoplastic transformation. It also raises the hope that reinvigorating such death susceptibility may reverse cancer cells to a hypersensitive state amenable to cure.

3. PHYTOCHEMICALS IN RESCUE: THEAFLAVINS LEADING THE ANTI-CANCER *OPERATION*

The above discussion suggests that targeting the tumor suppressor protein-p53 to induce wild type p53 functions or inhibit mutant p53 functions, by different phytochemicals may confer a high therapeutic index in tumors by simultaneously sensitizing the cancer clone and protecting the host, thus widening the index from both sides. In fact, a vast number of experimental studies convincingly show that many phytochemicals target p53 in cancer cells (14). Among these phytochemicals, black tea theaflavins, (3,4,5-trihydroxy-1,8-bis[(2R,3R)-3,5,7-trihydroxy-2-chromanyl]-6benzo [7] annulenone, $C_{29}H_{24}O_{12}$), have been identified as one of the major classes of natural anticancer agents exerting antineoplastic activity in various types of cancer cells. These black tea polyphenols, comprising of theaflavin, theaflavin-gallate, theaflavin 3-gallate and theaflavin 3-3'-gallate (Figure 1) as formed from catechins during the enzymatic oxidation of tea leaves (*Camellia sinensis*), have both immunostimulatory and anti-toxic properties (15-21). The inhibition of tumorigenesis by black tea and theaflavin preparations has been demonstrated in animal models on different organ sites such as skin, lung, oral cavity, esophagus, fore-stomach, stomach, small intestine, colon, pancreas, and mammary gland (22). Black tea infusion was also found to be effective against chemically and UV-induced tumorigenesis (23). Several such studies have discussed the anti-cancer effects of theaflavins in both cell line and animal models (22, 23). However, review on the target-based approach is still the Cinderella of discussion. This review elegantly emphasizes on the functional importance of both wild-type and mutant p53 in the process of carcinogenesis and concurrently highlights how theaflavins, by targeting p53 and/or modulating p53 functions, aid to cancer prevention (Figure 1).

4. P53: The guardian of genome

The *TP53* gene has a prominent role in cancer and much of human biology. The p53 tumor suppressor can be induced by a range of stresses through transcriptional (24), posttranscriptional (25) and post-translational (26) control mechanisms. The 'guardian of the genome' continues to fascinate investigators because of its many functions including direct roles in repair and recombination, association with proteins involved in genome stability, and chromatin modification (27). However, its broadest cellular effect is that of a transcription factor (28). In its role as a master regulator, p53, by cross talking in a large network of messengers and effectors, governs the complex multistep process of carcinogenesis, e.g., metabolism, apoptosis, cell cycle regulation, chemo and radiosensitization**,** angiogenesis and metastasis (27, 29, 30) (Figure 1). Because p53 plays nemesis by condemning tumor cells, p53 functions are often impaired either due to degradation following direct interaction with negative regulators of the p53 pathway or at times indirectly inhibited by different auxiliary proteins. Regardless of the precise mechanism by which the disruption of the p53 pathway induces immortalization, this pathway is frequently altered in nearly 50% of primary tumors and tumor-derived cell lines (31). In the remaining 50% of cancers site specific mutations in the p53 gene have resulted in altered p53 functions (32). Currently, around 11 million people are living with a tumor that contains an inactivating mutation of p53 and another 11 million have tumors in which the p53 pathway is partially abrogated through the inactivation of other signaling or effector components (33). Identification and characterization of molecular components important in both p53-dependent and p53-independent apoptosis might thus be useful in

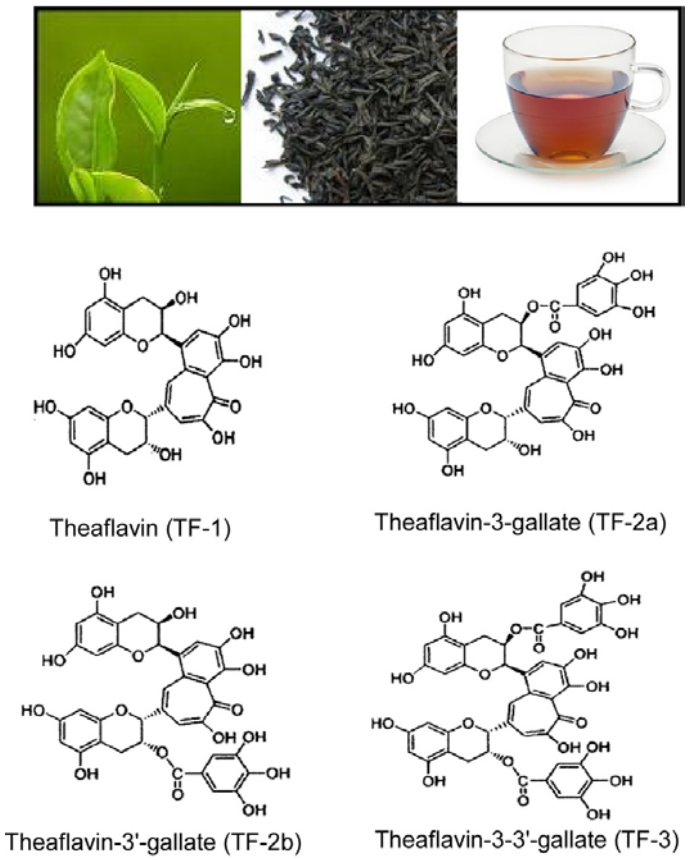

Figure 1. Structure of theaflavins: Photographic images of tea leaves and the structures of different theaflavin derivatives.

developing novel therapies. Increasing numbers of sequences obtained from human cancers add to a database of over 10,000 somatic tumorigenic *p53* mutations. Six "hot spots" are most frequently associated with cancer are Arg^{175}, Gly^{245}, Arg^{248}, Arg^{249}, Arg^{273}, and Arg^{282} (34). Accumulating evidence has indicated that p53 mutants not only lose tumor suppressor activity but also gain distinct oncogenic properties to promote the process of tumorigenesis (Figure 2). The p53 pathway is therefore a prime target for new cancer drug development.

Categorization of p53 functions under the following headings: (i) functional (wild-type) (ii) inactive (wild-type p53, but the activity is impaired because of its degradation and/or inactivation of auxiliary signaling or effector components) and (iii) mutant will, therefore, certainly illuminate our knowledge about p53's stand in carcinogenesis and at the same time highlight the possibility of targeting this master regulator.

4.1. Functional p53 'handcuffs' Cancer

The anti-tumor effects of wild-type p53 have been summarized below under the following headings:-

4.1.1. Tumor metabolism: p53 empties the fuel tank

Cancer cells have acquired distinctive characteristics that distinguish them from their normal counterparts and among the very first of these differences were the changes in tumor cell metabolism. For example, glucose uptake was found to be much higher in tumors than in most normal tissues, and the persistence of glycolysis even under normal aerobic conditions led Otto Warburg to propose that these metabolic changes were at the heart of cancer development — leading to, rather than resulting from, malignant transformation (35). In the intervening years, as the importance of oncogenes, tumour suppressors, proliferation and apoptosis was unravelled, the role of metabolism in cancer development became somewhat sidelined, with the general feeling that the metabolic changes were simply a by-product of malignant transformation. However, an increasing understanding of the molecular mechanisms that control metabolism has led to a resurgence of interest in understanding how metabolic transformation can have a crucial role in the maintenance of the tumorigenic state. The need for glycolysis or the reduced dependence on oxidative phosphorylation for efficient energy production shown by cancer cells is not generally due to a defect in components of the TCA cycle or the electron transport chain, but reflects an ability of proteins associated with oncogenic transformation to promote glycolysis. These include not only Akt and Myc but also other oncoproteins associated with deregulated proliferation (35). Several studies have shown that p53 has a role in the regulation of both glycolysis and oxidative phosphorylation (Figure 3). Numerous mechanisms have

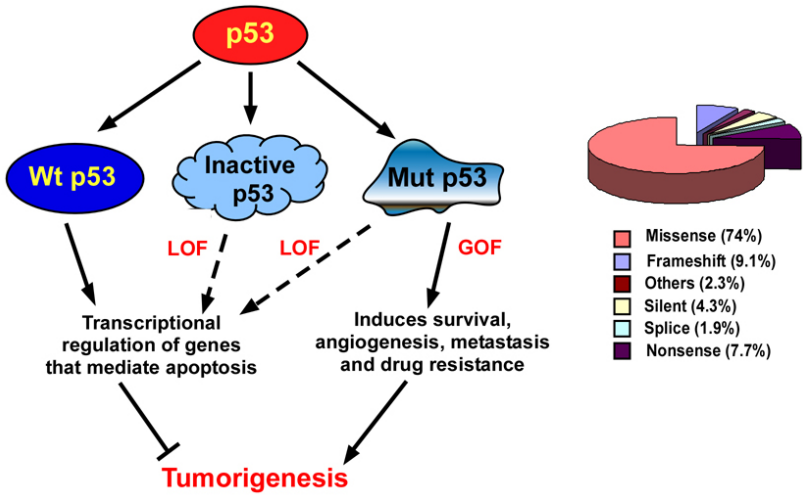

Figure 2. Categorization of p53 types and functions: Schematic representation depicting how Wt (wild type) p53, inactive p53 and mut (mutant) p53 regulates the process of oncogenesis. When compared to functionally active Wt p53, inactive p53 shows LOF (loss of function) while mutant p53 manifests both LOF and GOF (gain of functions) e.g., survival, angiogenesis, metastasis and drug resistance. Pie-chart representing the different tumor-derived mutation types.

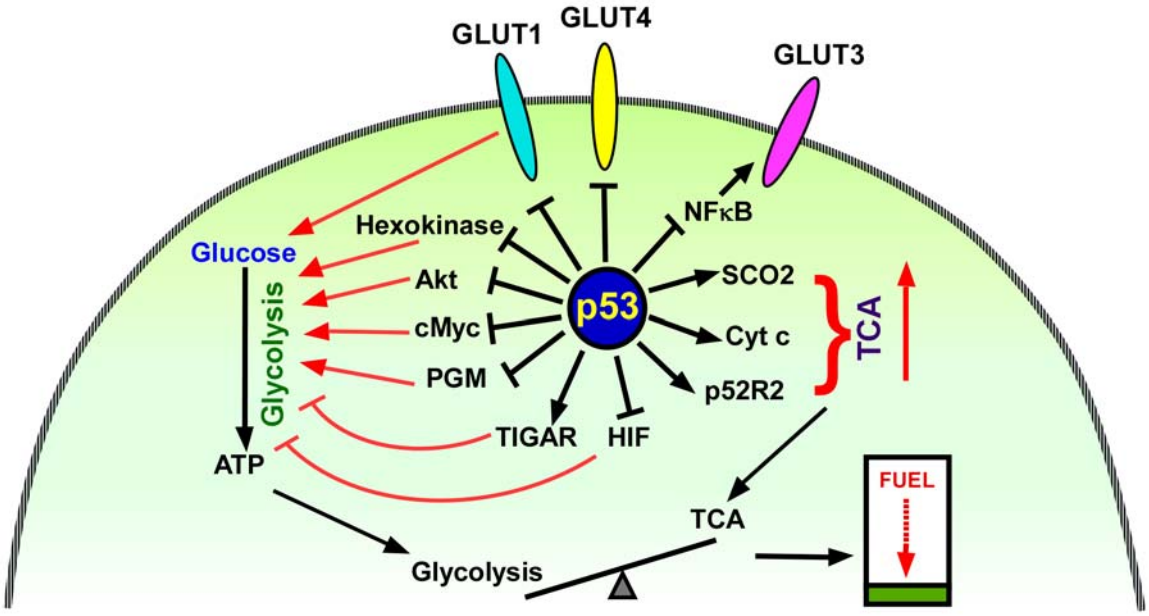

Figure 3. Functional p53 inhibits tumor glycolysis: Cancer cells depend on glycolysis, irrespective of oxygen conditions to meet their energy demands. Consequently, a shift from glycolysis to tricarboxylic acid cycle in tumor cells drastically reduces the net energy production, thereby making them vulnerable to cytotoxic effects of glycolysis inhibitors. Inhibition of glucose transporters like GLUT1, 3 and 4 by p53 restricts excess glucose entry, the substrate for the glycolytic pathway. p53 simultaneously inhibits the glycolytic promoting proteins like Hexokinase, Akt, cMyc, PGM and HIF while activates SCO2, Cyt c, p52R2 and TIGAR, proteins that induce mitochondrial respiration.

now been described through which p53 can slow glycolysis and therefore counteract the increase in glycolysis that is characteristic of cancers. In fact, p53 can inhibit the expression of the glucose transporters GLUT1 and GLUT4 and can decrease the levels of phosphoglycerate mutase (PGM) while increasing the expression of TIGAR (TP53-induced glycolysis and apoptosis regulator) (36-38). Expression of p53 can limit the activity of IKKalpha and IKKbeta, thereby restricting the activation of NFkappaB and dampening the expression of glycolysis-promoting genes such as GLUT3 (39). The exact mechanism by which p53 functions in this pathway is not clear but relates to the ability of p53 to oppose the activating O-linked beta-*N*-acetyl glucosamine modification of IKKbeta (39). The restraint on glycolytic rate imposed by p53 is paralleled by the ability of p53 to help maintain mitochondria and drive

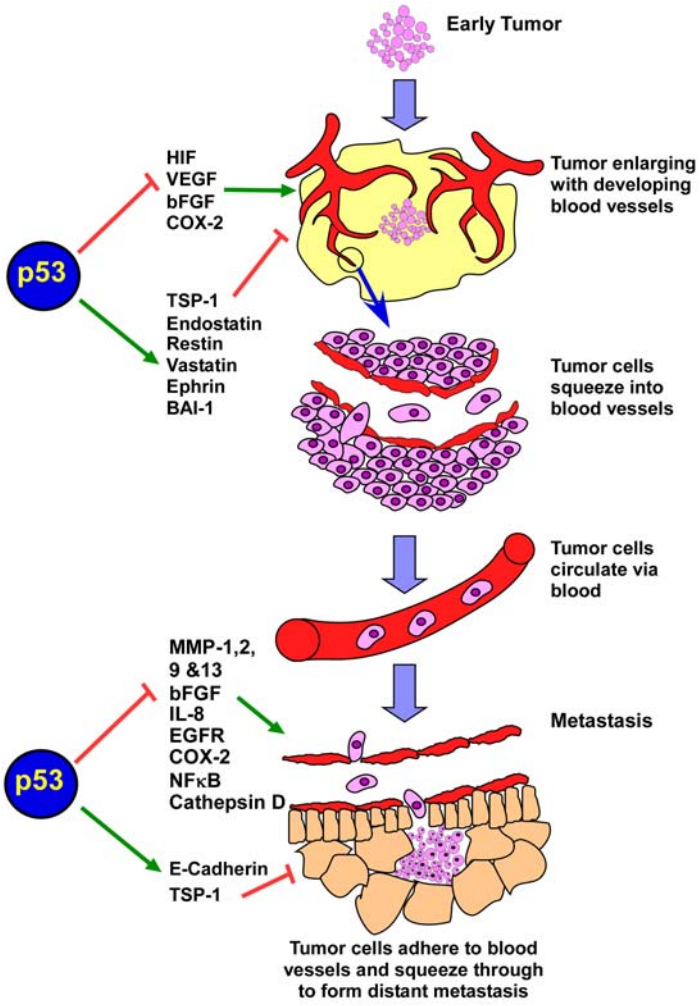

Figure 4. Functional p53 inhibits tumor angiogenesis and metastasis: Neovasularization around cancer cells is an important event during tumor angiogenesis. P53 inhibits tumor angiogenesis by abrogating the expression of factors that promote angiogenesis e.g., HIF, VEGF, bFGF and Cox-2. Concomitantly p53 promotes angiogenesis inhibition factors like TSP-1, endostatin, restin, vastatin, ephrin, BAI-1. p53 regresses metastasis by inhibiting MMP-1, 2, 9 & 13, bFGF, IL-8, EGFR, Cox-2, NFkappa B while inducing E-cadherin and TSP-1.

oxidative phosphorylation, and so represents another manifestation of the tumor-suppressive activity of p53. These effects are likely to be the consequence of several p53-dependent functions, including the transcriptional activation of subunit-I of cytochrome c oxidase (35), activation of expression of synthesis of cytochrome c oxidase-2 (SCO2), a key regulator of the cytochrome c oxidase complex, and induction of expression of the ribonucleotide reductase subunit p52R2, a protein that contributes to the maintenance of mitochondrial DNA (35). The ability of p53 to promote oxidative phosphorylation is also demonstrated by the effect of reducing expression of cytoplasmic polyadenylation element-binding protein (CPEB), a protein that increases translation of mRNA by promoting polyadenylation (40). One of the targets of CPEB is *TP53* mRNA, and cells with reduced CPEB levels express only half the normal levels of p53 (40). Intriguingly, the reduction of p53 expression is accompanied by a switch from oxidative phosphorylation to glycolysis. It is clear that alterations in metabolism can have a role in cancer development, and that p53 can regulate various aspects of metabolism. Although the implications of these two statements are tantalizingly obvious, fitting the metabolic activities of p53 into a simple paradigm of how cancers are regulated is less straightforward.

4.1.2. Angiogenesis: p53 blocks fuel supply

Angiogenesis is the physiological process of generations of new blood vessel capillaries. The process of angiogenesis is tightly synchronized in an intricate manner by a balance between pro- and anti-angiogenic factors, which ultimately governs the “angiogenic switch” of cells. Since angiogenesis is most dynamic in rapidly growing tissues (e.g., a malignant tumor), growth-promoting genes (i.e., oncogenes) frequently up-regulate the progression of

angiogenesis and, conversely, tumor suppressor genes like p53 often negatively regulate angiogenesis (41). The anti-angiogenic effect of p53 is depicted by clinical studies demonstrating that tumors with p53 mutations are highly vascularized compared to tumors with wild-type p53. The microvessel density, a semi-quantitative measure for scoring tumor vascularization, is greater in prostrate cancer, colon cancer, head and neck tumors and breast cancer with p53 mutations (42-45). In recent years numerous research programs are aiming towards understanding the linkage between p53 and regulatory pathways of angiogenesis (Figure 4). These studies have defined three basic mechanisms by which p53 inhibit angiogenesis: (i) inhibition of hypoxia sensing system, (ii) down-regulation of pro-angiogenic factors, and (iii) up-regulation of anti-angiogenic signaling pathways. HIF-1alpha, a component that responds to oxygen deprivation, is the principal transcriptional activator of the main pro-angiogenic genes like VEGF and bFGF (46). p53 not only inhibits HIF-1alpha activity by directly binding it and targeting the protein for degradation by ubiquitination but also directly represses the expression of several pro-angiogenic genes including VEGF, basic fibroblast growth factor (bFGF), bFGF-binding protein (bFGF-BP), and cyclooxygenase-2 (COX-2) (47-51). VEGF expression in hypoxia is inhibited by p53 by binding the transcription factor SP-1 and limiting its capability to bind the VEGF promoter and activate VEGF transcription (48). Likewise, p53 represses expression of bFGF through direct repression of the bFGF basal core promoter, in addition to expression of the bFGF-BP gene, which activates bFGF (49-50). COX-2 repression by p53 is carried out by a mechanism where p53 competes with TATA-box binding protein for binding to the COX-2 promoter (51). Anti-angiogenic factors which are up-regulated by p53 are, in most cases, factors that are secreted into the extracellular matrix (ECM). One of the first target genes activated by p53 was thrombospondin-1 (TSP-1), which was also the first endogenous factor found to inhibit angiogenesis (52). p53 also activates brain-specific angiogenesis inhibitor 1 (BAI1) that is a large transmembrane protein of the B family of G-protein-coupled receptors and was originally identified in glioblastoma cells (53). Ephrin signaling is another anti-angiogenic response up-regulated by p53 (54). Ephrin receptor A2 (EPHA2) is a member of the largest family of receptor tyrosine kinases called the ephrin receptor which together with their ligands (ephrins) are believed to play important roles in angiogenesis (55). p53 also stimulates production of several potent anti-angiogenic factors such as Endostatin, Restin, Vastatin (56-58).

4.1.3. Metastasis: p53 halts the race

Tumor metastasis is the main cause of mortality and treatment failure in cancer patients. Biochemically, metastasis results from alterations in expression of many genes, the well-documented ones include epidermal growth factor receptor (EGFR) for growth; basic fibroblast growth factor (bFGF), interleukin-8 and thrombospondin (TSP-1) for angiogenesis; metalloproteinase (MMP)-2, MMP-9, and cathepsin D for invasion; E-cadherin for adhesion; and multidrug resistance-1 (MDR-1) for drug resistance. Increased expression of EGFR, bFGF, interleukin-8, MMP-2, MMP-9, cathepsin D, and MDR-1 and decreased expression of E-cadherin and TSP-1 are associated with metastasis promotion (59). It has been appreciated that p53 regulates expression of some metastasis-related genes. This tumor suppressor can transcriptionally inhibit the expression of MMPs, EGFR, bFGF and MDR-1, while inducing metastasis suppressor genes TSP-1 and KAI1 (60-66). Reintroduction of wild type p53 into STS xenografts decreased tumor growth and MMP-9 protein expression by inhibiting NFkappaB function (60-66). p53 also inhibits metastasis by inducing genes that block ECM degradation and by repressing genes that degrade the ECM (67, 68). p53 induces two serpins, plasminogen activator inhibitor-1 (PAI-1) and maspin (67, 68). PAI-1 limits the metastatic potential of tumors by inhibiting urokinase-type plasminogen activator (u-PA), which initiates a cascade of cleavages that result in the activation of plasmin that degrades a wide variety of ECM proteins, such as fibrin, fibronectin, and laminin (67). Over-expression of maspin in a highly invasive mouse mammary tumor has been found to inhibit tumor growth and metastasis (68). Recently, we have shown that p53 activation inhibited NFkappaB-mediated expression of MMP-2 and MMP-9 in a ROS-dependent manner thereby retarding human breast cancer migration (69).

4.1.4. Apoptosis: p53 bringing the end nearer

It is well-appreciated that apoptosis plays an important role in the process of tumor suppression (70). Although the ability of p53 to trigger cell-cycle arrest was discovered first, its action in controlling apoptosis is the most intensely studied. It was shown that oncogenes activate p53 tumor suppressor gene leading to apoptosis, and that p53 is required for apoptosis induced by certain DNA damaging anticancer agents (71). p53 acts at multiple levels of the intrinsic and extrinsic death pathways *via* the induction of multiple pro-apoptotic target genes as well as *via* transcription-independent mechanisms (72, 73) (Figure 5). In most cases p53 activates several important genes that are crucial for the execution of the intrinsic apoptotic pathway including pro-apoptotic genes such as Bax, Noxa, PUMA, and Apaf–1 (74-77). Furthermore, p53 represses the apoptosis repressor with caspase recruitment domain protein, which counteracts the apoptotic functions of PUMA and Bad (78). p53 can also promote cytochrome c release by inducing the expression of the OKL38 tumor suppressor gene, which localizes to the mitochondria and augments cytochrome c release (79). Silencing OKL38 correlates with tumorigenesis, and its over-expression induces apoptosis in several carcinoma cell lines (79). In the extrinsic pathway, p53 induces the expression of the death receptor Fas and DR5 as well as of TRAIL death ligand and the Fas ligand (80). In addition to its activity as a transcriptional regulator, p53 can also induce cell death in a transcription-independent manner. It has been shown that p53 is able to physically interact with several anti-apoptotic proteins including Bcl-2, Bcl-xL and mcl-1 at the mitochondrial membrane thereby resulting in mitochondrial membrane permeability changes and release of cytochrome c (81). Additionally, p53 can interact with Bak and thus directly induce the release of cytochrome c from the intermembrane space of the mitochondrion (81). Studies

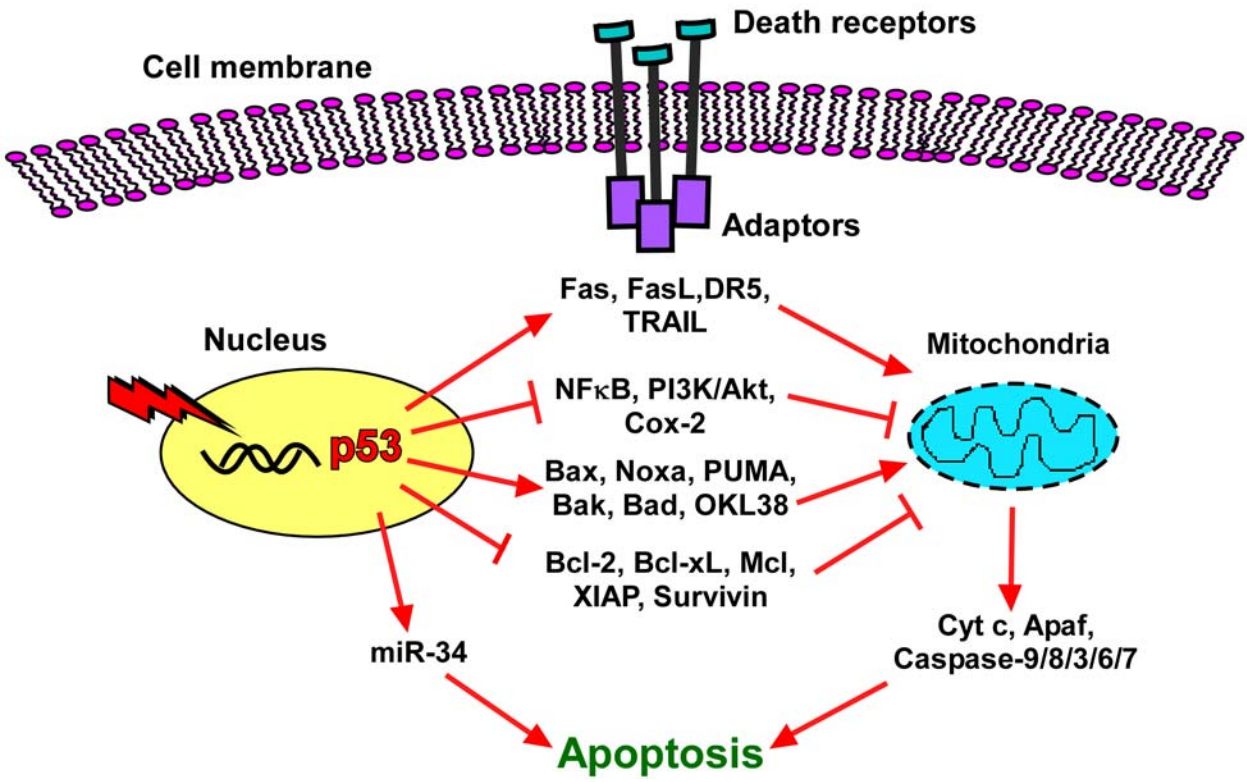

Figure 5. Functional p53 triggers tumor apoptosis: Evasion of the cell death programme is one of the major hallmarks of neoplastic cells. p53 by inducing the process of apoptosis in tumor cells sensitizes tumors to conventional therapies. DNA damage activates p53 which in turn triggers the intrinsic cell death machinery by inducing pro-apoptotic proteins e.g, Bax, Noxa, PUMA, Bak, Bad and OKL38 while inhibiting anti-apoptotic proteins e.g, Bcl-2, Bcl-xL, Mcl, XIAP and Survivin. This leads to disruption of mitochondrial membrane potential causing release of mitochondrial cytochrome c, formation of Apaf-1 complex and activation of initiator and executioner caspases. p53 also activates the extrinsic cell death cascade by inducing the expression of death receptors (FAS, DR5) and their ligands (FASL, TRAIL). In addition p53 inhibits the major cell survival pathways e.g, NFkappaB, PI3K/Akt and Cox-2. P53 also induces miR-34, a new participant of the apoptosis programme.

demonstrate that repression of Rel A by p53 through a p300-dependent mechanism counter attacked the well-accepted theory of NFkappaB-mediated oncogenesis (82). Interestingly, stress-induced activation of p53 also counteracts the inhibitory effects of another survival pathway, Akt, by multiple mechanisms (72). First, p53 promotes caspase-mediated cleavage and subsequent degradation of the Akt protein itself (72). Second, p53 induces the expression of the *PTEN* tumor suppressor gene, which encodes a phosphatase that dephosphorylates PI3K, thereby impairing Akt activation (83). Lastly, it was recently shown that p53 regulates the expression of microRNAs (miRNAs), where a principal role has been accredited to the miR-34 family (84). Inactivation of miR-34 attenuates p53-mediated apoptosis in cells exposed to genotoxic stress, suggesting a role for this microRNA in regulating p53 responses (84).

4.1.5. Drug resistance and cancer therapeutics: p53 overcomes the challenge

There is evidence that the status of p53 in tumor cells is an important determinant not only of tumor development, maintenance and progression, but also of its therapeutic response (85). In early studies, wild-type p53 was defined as a treatment sensitivity factor promoting chemotherapy- or radiotherapy-induced apoptotic cell death of tumor cells (86). Additional evidence linking p53 status and response to therapy concluded that the vast majority of clinically used chemotherapeutic agents are more effective in killing human tumors with wild-type as compared to mutant p53 (87, 88). Using two models of experimental tumors with wild-type p53 (i) mouse embryo fibroblasts that were transformed *in vitro* by the combination of adenoviral protein E1A and mutated oncogene H-Ras and (ii) transgenic mice that carry the c-Myc oncogene that develops spontaneous mouse lymphomas, it has been shown that transformed cells respond to DNA damage with p53-dependent apoptosis, and suppression of p53 results in tumour resistance to treatment (89, 90). The work of Lowe *et al*. further reported that wild-type p53-expressing mouse thymocytes or mouse embryonic fibroblasts expressing Ras and E1A were much more likely to undergo apoptosis following exposure to cytotoxic chemotherapeutic agents or ionizing radiation (91). More recent studies by Lowe and colleagues have elegantly demonstrated that p53-deficient lymphoma cells are slow to respond to cytotoxic therapy and invariably relapse and confer a poor survival to the mice (92). On the other hand, lymphomas that carry wild-

type p53 but also express the antiapoptotic protein Bcl2 are also less responsive to chemotherapy, but ultimately the mice have a better survival due to the cell cycle arrest and senescent programs activated by p53 when their lymphoma cells are exposed to cytotoxic therapy (89). Evidence for an association between loss of wild-type p53 and failure to respond to chemotherapy has also been obtained for ovarian cancer (93). p53 aberrations may also predict failure to respond to cisplatin-based chemotherapy in non-small-cell lung cancer (94). Wild-type p53 is therefore thought to make tumors more sensitive to treatment through the induction of apoptosis, whereas p53 inactivation or loss of p53 function is thought to lead to treatment resistance with an unfavorable prognosis in many forms of cancer. Consequently, the p53 gene has been a major candidate for somatic gene-therapy approaches to human cancer, often with the goal of reconstituting response to radiotherapy and chemotherapy. In principle, p53 may enhance chemosensitivity by promoting apoptosis via transcription-independent mechanisms as well as transcriptional activation of proapoptotic genes such as Bax and transcriptional repression of anti-apoptotic genes such as Bcl-2 (95). Drug-induced suicide mediated by the CD95/CD95 ligand system may also involve a p53-controlled pathway (95). Importantly loss of wild-type p53 activity and acquisition of a multidrug resistance (MDR) phenotype, two key factors in the resistance of human cancers to chemotherapy, may be interrelated. Wild-type p53 represses expression of both the MDR-1 genes (encoding the prototypical MDR protein P-glycoprotein [PGP]) and MRP-1 (encoding the MDR-associated protein) (96). Wang *et al.* further demonstrated that wild-type p53 acts as a negative regulator of MRP gene transcription, at least in part by diminishing the effect of a powerful transcription activator Sp1 (97). Therefore, a loss of p53 function and/or an increase in Sp1 activity in tumor cells could contribute to an up-regulation of the MRP gene. All these information will be useful in developing strategies to address the problems of multidrug resistance.

4.2. Inactivation impairs wild-type p53 functions

That the loss of wild-type p53 function is directly associated with escape from senescence was initially demonstrated through the introduction of a dominant-negative p53 gene in fibroblasts that permitted those cells to proliferate for a limited number of population doublings, beyond the point at which their normal counterparts become senescent (31). Evidence that these was attributable to loss of wild-type p53, and not gain of p53 function, was provided by studies with Li-Fraumeni fibroblasts (31). A substantial effort is presently focused on developing means for activating p53 functions in tumors harboring repressed wild-type p53, by inhibiting its interaction with MDM2 or by functional inhibition of ubiquitine, proteosomes and other cellular effectors that down-regulate p53 level or function (98). p53 specific E3 ubiquitin ligase-MDM2 and MDM4, known to enhance tumorigenic potential and resistance to apoptosis, have been reported to be over expressed in different cancers including sarcoma, brain tumors, and lung cancer (99). Furthermore amplification of MDM-2 has been correlated to metastasis and disease recurrence in osteosarcoma patients (100). Concurrently deregulation of p19/ARF-p53 pathway that negatively regulates MDM-2-mediated p53 degradation is frequently observed in large majority of human tumors (101). Other proteins that act as E3 ligases to promote p53 degradation include PIRH2, which is a p53 target gene (102). Katayama and colleagues recently showed that aurora kinase A, which is frequently over expressed in bladder and other human cancers, phosphorylated p53 at serine 315, resulting in its destabilization and degradation (103). Another recent report by Pohler and colleagues indicates that Barrett's epithelium, a pre-malignant oesophageal lesion, overexpresses anterior gradient 2 (AG2) and is associated with a marked decrease in ultraviolet (UV)-light-induced p53 transactivation and phosphorylation at Ser-15 and Ser-392 (104). Loss of ATM, an important stabilizer cum activator of p53, has also been demonstrated in substantial cases of chronic lymphocytic leukemia. Some authors have postulated that in patients with low ATM levels, p53 may not be sufficiently activated for induction of apoptosis in response to DNA-damaging agents (105). Other reason why p53 is functionally compromised in cancers is because of ΔTA-p73 which acts as a dominant-negative inhibitor of p53 (106). Further up-regulation of endogenous p73 just like ectopic overexpression of ΔTA -p73 confers resistance to p53-mediated apoptosis induced by the chemotherapeutic agent (106). Additionally several tumor-inducing human viruses, including certain small DNA viruses (adenoviruses, polyomaviruses, papillomaviruses 16 and 18, and hepatitis B and C viruses), large DNA viruses (cytomegalovirus, herpes virus 6 and 8, Epstein-bar virus) and human retroviruses (HTLV-1 and HTLV-2), adopted various mechanisms for p53 inactivation by their virally encoded oncoproteins as part of their oncogenic potential (107). Cumulatively impairment of functional p53 (Figure 6) is a major contributor in neoplasia that as well functions as a barrier to p53 based drug therapies.

4.3. Mutant p53: When the savior becomes the slayer

Just as wild-type p53 continues to surprise us by having roles in a much broader range of processes than previously thought, interesting new functions for mutant p53 are also being uncovered (Figure 2). A summary of the gain of function (GOF) properties of mutant p53 has been elaborated below.

4.3.1. Mutant p53 sustains energy

The connections between cell proliferation and increased glycolysis in cancer cells were first made by the demonstration of mutant p53 transactivating the hexokinase 2 gene in hepatoma cells (108). Subsequently, another glycolytic enzyme PGM was shown to be induced by mutant p53 and important for immortalizing mouse embryo fibroblasts (109). Thus it appears that mutant p53, by favoring the expression of glycolytic enzymes, meets the surplus energy demand in malignant cells. However, more works are needed to get detail information regarding the role of mutant p53 in tumor metabolism.

4.3.2. Mutant p53 supplies fuel in excess

Loss of p53 corresponds to a turning point in which small dormant tumors undergo an angiogenic switch

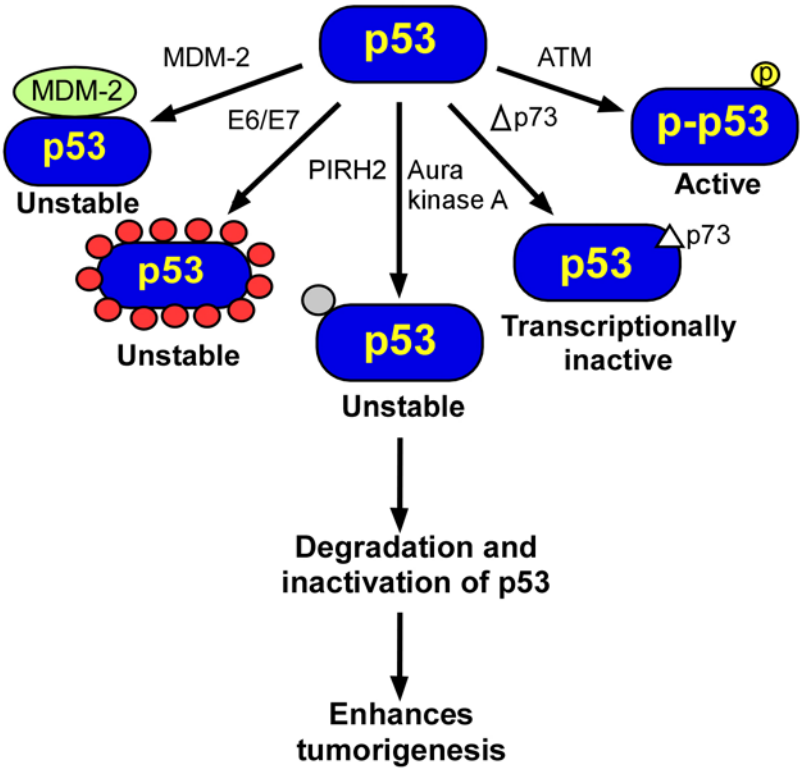

Figure 6. Impairment of functional p53: P53 is targeted to inactivation in a wide range of tumors via alternate mechanisms. P53 (i) undergoes destabilization upon binding to MDM-2, PIRH2, aurokinase A and HPV E6/E7 oncogene, (ii) when bound to delta p73 it undergoes transcriptional inactivation and (iii) loss of ATM leads to p53 de-phosphorylation and inactivation.

and aggressively begin to inflate due to neoangiogenesis (110, 111). This model is supported by two observations: first, reversal of the angiogenic switch requires either p53 or TSP-1 expression, and second, p53 expression has been shown to induce tumor dormancy in several systems by limiting angiogenesis (110, 111). It has also been shown depletion of mutant p53 protein severely impairs ID4 (inhibitor of DNA binding 4) expression in proliferating tumor cells (112). ID4 is a member of a family of proteins that function as dominant-negative regulators of basic helix-loop-helix transcription factors and is linked to cell proliferation, differentiation and tumorigenesis (112). On the other hand presence of p53 hot-spot mutations at 175 (R175H), 248 (R248W), and 273 (R273H) amino acids increases the vascularization inside the tumors by several folds (113). In gist, presence of mutant p53 initiates the risk of turnover of a small benign tumor to an invasive malignant tumor.

4.3.3. Mutant p53 accelerates the race

Adorno *et al.* describe a new pathway that suggests opposing roles for mutant p53 and uncover a gene signature that could be clinically useful for predicting breast cancer prognosis (114). In MDA-MB-231 breast cancer cells which express endogenous mutant p53 (p53R280K), TGF-beta 1 induced migration that was dependent on mutant p53 expression and canonical SMAD signaling (114). Mutant p53 was also required for lung colonization following intravenous injection of MDA-MB-231 cells. Stable reconstitution of TP53-null H1299 lung cancer cells with the p53 R175H hotspot mutation, combined with TGF-beta 1 treatment, induced a mesenchymal phenotype and *in vitro* migration (114). Furthermore, decreased expression of E-cadherin is associated with p53 alternations such as point mutations and protein accumulation (115).

4.3.4. Mutant p53 overpowers death

The combined *in vitro* and *in vivo* data suggest that when some mutant p53 forms accumulate, their oncogenic properties are enhanced, prompting the careful consideration of p53□activating drugs when treating tumors that express mutant p53 (116). It has been well-documented that p53 mutants differ in their ability to resist apoptotic stimuli. Specifically, the R175H hot spot mutant has been shown to be a potent inhibitor of apoptosis (117). It has been suggested that the R175H mutation limits the flexibility of p53, thereby "locking" p53 into a more rigid conformation that reduces its DNA-binding capacity and possibly the ability to interact with other proteins. Hence, the mechanism whereby the R175H mutant resists apoptotic stimuli is attributed to reduced transcriptional up-regulation of apoptotic intermediates. Several cancer-associated p53 missense mutants are required for the survival of breast cancer cells (118). For example, inhibition of endogenous mutant p53 by RNAi led to massive apoptosis in two mutant p53-expressing cell lines, T47D and MDA-MB-468, but not in the wild-type p53-

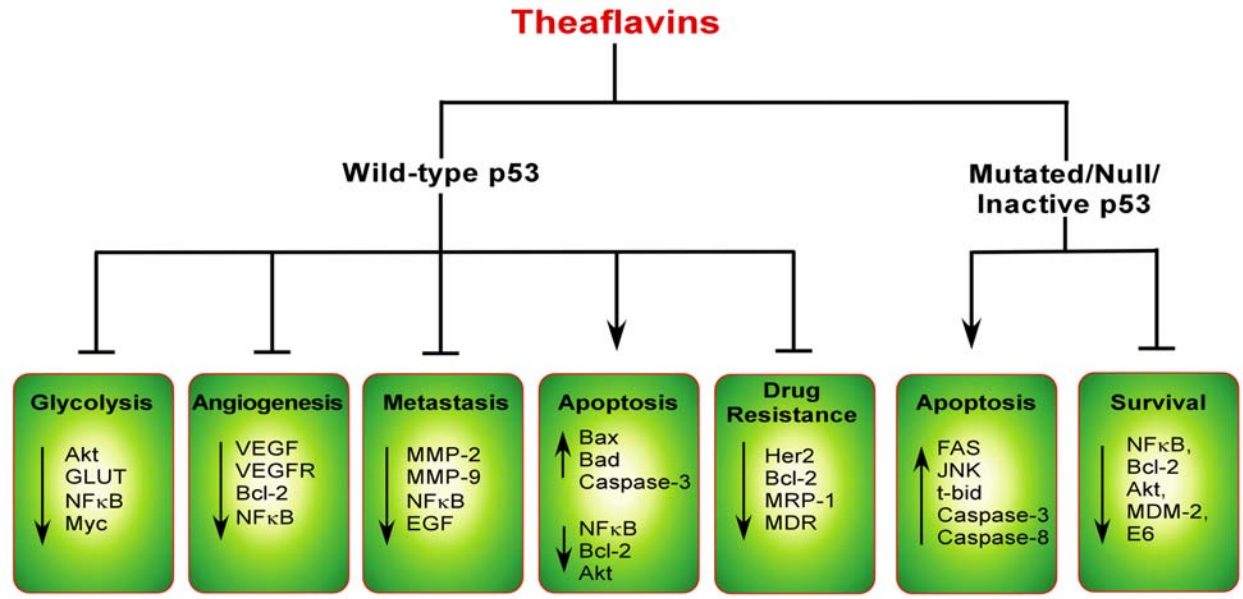

Figure 7. Anti-cancer targets of theaflavins: Theaflavins abrogate the process of tumorigenesis both in presence and absence of wild-type p53. In presence of p53, theaflavins target p53 to inhibit glycolysis, retard angiogenesis and metastasis, induce apoptosis and overcome drug resistance in cancers. In the process p53 modulates different key molecules like Akt, Bcl-2, Bax, caspase-3, Her2, MMP, NFkappaB, p38, ROS and VEGF/VEGFR. In absence of wild-type p53, theaflavins check tumorigenesis by p53-independent alternative pathways e.g, Fas/caspase-8, JNK, Akt and NFkappaB. When inactivated due to degradation, theaflavins induce p53 stabilization through inhibition of MDM2 and E6.

expressing cells, MCF-7 and MCF-10A (118). A search for the mechanisms revealed that mutant p53 gains anti-apoptotic function through signal transducer and activator of transcription 3 in anaplastic thyroid cancer cells (119).

4.3.4. Mutant p53 strengthens drug resistance

The effect of mutant p53 on drug resistance has simultaneously been under intense investigation. According to Lowe *et al.* (120), mutant p53 favored resistance to a variety of anticancer agents through interference with apoptosis. While Chin *et al.* (121) demonstrated that mutant p53 specifically stimulated the MDR1 promoter thereby implying that the MDR1 gene could be activated during tumor progression associated with mutation in p53. Zhang *et al.* demonstrated the association of p53, P-glycoprotein (PGP) and Glutathione S-Transferase-pi (GST-pi) with multi-drug resistance of colorectal carcinoma, and that the overexpression of PGP and GST-pi was closely correlated with mutant p53 (122).

With thousands of such loss and alterations of p53 functions identified in tumor patients, how can future cancer therapy buttress this fragile protein structure and restore the cell's natural defense?

5. THEAFLAVINS EXPLOIT P53 DURING THE ANTI-CANCER *OPERATION*

Consequently, the challenge has been to identify compounds that can induce, re-install functional p53 and/or revert mutant p53 dysfunctions to combat tumorigenesis in cancer patients. Theaflavins as foresaid, by exploiting p53 when present or bypassing when absent, are reported to execute strong anti-carcinogenic effects

5.1. Theaflavins in presence of functional p53

In cancer there are believed to be specific alterations to normal cell physiology, which together define the progression of most human malignancies. Research, from last few decades, highlights that all these hallmarks of cancer are inter-digitated with p53 as the pivotal molecule. The ultimate cancer therapeutic agent should, therefore, be the one that attends the master regulator of the genome with the aim of preventing, slowing down, or reversing the transformation process. In this review, we attempt to provide evidence for the preventive and therapeutic effects of black tea polyphenol, theaflavins highlighting the comprehensive state-of-the-art knowledge regarding their targeted effects on p53 in tumor cells (Figure 7).

5.1.1. Depriving of currency for growth

Our effort to understand the role of theaflavins in cancer metabolism revealed that these polyphenols inhibit some of the mediators of glycolytic pathways e.g., IKKbeta, NFkappaB, Akt and GLUT (69, 123, 124). Since these proteins are also repressed by p53, which is activated by theaflavins, it may not be irrelevant to perceive that by targeting p53 these tea polyphenols are modulating tumor glycolysis (68, 125). It can thus be envisaged that theaflavins, by the virtue of its ability to activate p53, are also contributing in depriving the cancer cells from their currency for growth and development i.e., glycolysis. However, the reports directly describing that theaflavins inhibit the process of oncogenesis by regulating tumor metabolism are still scanty.

5.1.2. Inhibiting nutrient supply

Theaflavins have also been acknowledged to block angiogenesis leading to a starvation of the tumor (126). The significance of theaflavins-p53 cross-talk in inhibiting angiogenesis is lime lighted by the fact that these black tea polyphenols decrease VEGF production and VEGFR phosphorylation, a downstream effector of p53, reflecting inhibition of the kinase activity of VEGFR-2 in athymic nude mice implanted with tumor cells (48, 127, 128). In athymic nude mice implanted with androgen-sensitive human prostate cancer cells (CWR22Rnu1), angiogenesis was again inhibited by black tea and theaflavins *via* decrease in VEGF (129). Moreover theaflavins *via* p53 inhibit Bcl-2, the oncogene known to increase the angiogenic potential of tumor cells by enhancing the transcription of VEGF (130, 131). Prevention of angiogenesis in DMBA-induced oral cancer further provided a mechanistic basis for the chemopreventive potential of black tea polyphenols (132). In addition tea polyphenols inhibit the recruitment and/or activation of phagocytes of the innate immunity by suppression of IkappaB/NFkappaB, possibly *via* p53 thus reducing inflammation that may account for an indirect role in impeding angiogenesis (69, 133).

5.1.3. Halting the parade

Multiple evidences suggest that p53 pathway cross talks with almost all the important metastatic pathways, and therefore, absence of p53 promotes while mutant p53 drives metastasis (134). Thus targeting the signaling pathways of cell migration centering p53 is another important approach of cancer therapy. It was reported that theaflavins inhibit the development of metastases by inactivation of the proteolytic enzymes (126). Theaflavins and theaflavin digallate, were also found to inhibit invasion of highly metastatic mouse Lewis lung carcinoma cells and in 3-methylcholanthrene induced solid tumor model in mice by inhibiting MMP-2 and MMP-9 (135). These black tea polyphenols also induce a restrictive effect on malignant invasion of human stomach and colon cancer cells through their effect on urokinase and matrix metalloproteinases (136). Moreover, theaflavins have been found to inhibit EGFR signaling and EGF-induced anchorage independent cell transformation (137). The hypothesis that these effects of theaflavins are mediated through the activation of p53 has been proved not only by our recent study which revealed that these phytochemicals retard breast cancer cell migration by activating p53/ROS/p38MAPK positive feed back loop thereby resulting in inhibition of NFkappaB and pro-migratory enzymes MMP-2 and MMP-9, but also by the reports that MAP kinase-dependent pathways help to regulate p53 levels by regulating the expression of p53 mRNA and that p53, which is activated by theaflavins, can transcriptionally inhibit the expression of MMPs and EGFR (60-62, 69 138).

5.1.4. Targeting the absence

Multiple *in vitro* and *in vivo* evidences suggest that theaflavins also induce apoptosis in cancer cells by directly modulating p53 signaling pathways and/or interacting with a wide variety of p53 downstream signaling proteins and modifying their expression and activity (123, 125, 139-141). These black tea polyphenols could successfully inhibit proliferation of human epidermoid carcinoma and melanoma cells through augmentation of Bax:-Bcl-2 ratio, p53 and p21 and simultaneous inhibition of phospho-Akt (142). Theaflavins induce apoptosis in human prostate carcinoma cells via modulation of two related pathways: up-regulation of p53 and down-regulation of NFkappaB activity, causing a change in the ratio of pro- and anti-apoptotic proteins leading to apoptosis (143). A report from our laboratory already established the relationship between p53 status, p21 induction, Bcl-2/-Bax ratio, cell cycle deregulation and apoptosis in black tea-treated tumor cells (130)**.** Recent findings from our laboratory have revealed that theaflavins induce breast cancer cell apoptosis in a p53-mediated Bax transactivation-dependent manner through loss in mitochondrial transmembrane potential, release of cytochrome c and activation of death cascade (125). Other observations indicate that theaflavins can restrict benzopyrene-induced lung carcinogenesis by differential modulation of the expression of p53 and its associated genes such as Bax, Bcl-2, MDM2, p21 and p27, along with H-Ras, c-Myc and cyclin D1 (144). All these information highlight the fact that theaflavins target p53, augmenting the transcription factor to work as tumor suppressor in cancer cells thereby finally helping the cells to regain what was absent in them – the program of apoptosis

5.1.5. Overpowering disobedience

Resistance to plethora of chemotherapeutic drugs is a persistent problem faced in the treatment of cancer patients. Theaflavins that target p53, the tumor-suppressor essential for overcoming drug resistance, have become candidates of choice. Theaflavins attenuate tamoxifen resistance in HER2/Neu transfected human breast cancer cells through suppression of tyrosine kinase phosphorylation (145). In support to this, studies by Huang *et al.* imply that wild-type p53 limits survival of ErbB2-overexpressing breast cancer cells (146). These findings suggest the use of black tea polyphenols by exploiting p53 may be beneficial in the chemoprevention of hormone-dependent breast tumors and represent a possible remedy to overcome hormonal resistance of hormone-independent breast tumors (145). Black tea polyphenols inhibit Bcl-2 and Bcl-xL thereby sensitizing tumorigenic cells to chemo and radiation therapy (147)**.** These effects of theaflavins might be due to activation of p53 since it is acknowledged that p53 enhances chemosensitivity by promoting apoptosis via transcriptional activation of pro-apoptotic genes such as bax and transcriptional repression of anti-apoptotic genes such as Bcl-2 (148). Since wild-type p53 represses expression of different drug transporters, theaflavins, by virtue of activating p53, might also be contributing indirectly in decreasing the multi-drug resistance of the cells (125). Also, an increase in p53 function in tumor cells, as is induced by theaflavins, could contribute to the down-regulation of the MRP gene, known to be repressed by p53 (125). Reports where theaflavins sensitize and induce apoptosis in drug resistant lung cancer cells by attenuating telomerase may possibly involve p53 since reports indicate that p53 directly inhibits telomerase activity, independent of its effects on cell growth arrest, cell cycle arrest, and

apoptosis (149, 150). However, more in depth study is required to understand the direct role of theaflavins in utilizing p53 to overcome drug-resistance in cancer cells.

These findings suggest that theaflavins, by regulating different aspects of tumorigenesis *via* p53, may be beneficial in the chemoprevention of different cancers.

5.2. Theaflavins in absence of functional p53

Principal mechanisms of drug resistance are over expression of drug efflux pumps, enhanced DNA repair and failure to apoptose as a result of mutation in proteins such as p53. Because p53 is frequently mutated in cancer, agents that preferentially kill p53-null cells and protect wild-type p53-expressing cells are highly desirable chemotherapeutic agents (151). Since p53 facilitates the anti-carcinogenic effects of theaflavins, its absence in cancers would appear to impose resistance not only to drugs-in-use but also to theaflavins. However, treatment of human leukemic p53-null cells, HL-60 with theaflavins, showed a dose dependent inhibition of growth and suppression of cell proliferation (152). Interestingly, theaflavins utilize caspase-8/t-Bid and Akt/Bad pathways in p53-null or -mutated cancer cells indicating their ability to induce apoptosis bypassing p53 pathway (123). Additionally, our recent findings indicate that theaflavins stabilize p53 in virally infected cervical cancer cells, manifesting lack of p53 owing to viral oncoprotein E6-mediated degradation, by inhibiting viral protein E6 and MDM-2 and thereby stimulating p53-dependent apoptosis (unpublished data). However, introduction of *p53* gene alone fails to trigger the apoptotic programme in p53-null cancer cells thereby explaining reasons of the failure, as in some cases, of p53 gene therapy in cancer (125). In these p53-engineered cells, theaflavins not only induce p53 expression but also efficiently trigger p53-dependent apoptotic cascade (125).

All the above reports clearly demonstrate how theaflavin modulates functional p53 when available, activates/stabilizes when inactive and finds an alternate way even in absence of functional p53 to carry forward the process of apoptosis in different tumors (Figure 7).

6. CANCER STEM CELLS: P53 BREAKING DOWN TUMOR'S ENGINE

The recently popularized 'cancer stem cell' (CSCs) hypothesis questions whether all or few tumor cells can participate in tumor evolution and restricts this property to a subset of them defined as 'cancer stem cells' due to their stem cell-like characteristics. According to this theory, tumorigenesis and its maintenance are driven by a limited subpopulation of tumor-initiating cells termed 'cancer stem cells' or referred as the 'tumor's engine'. CSCs retain stem like properties such as the ability to self renew, increased proliferative capacity, and the ability to differentiate into different lineages. These cells have been posited to be responsible for resistance to conventional therapies and metastasis, leading to tumor relapses and contributing to poor prognosis for cancer patients. CSCs were first identified in the hematopoietic system and subsequently in a variety of solid tumors including brain, breast, colon, prostate, and others (153). Previous studies demonstrate that mutations in the *p53* gene are one of the early premalignant events in the majority of human malignancies (154). Additionally, Meletis *et al.*, demonstrated a p53-mediated suppression of neural stem cell proliferation and self-renewal in a mouse model (155). Interestingly, stem cells with targeted mutation of the tumor suppressor p53 possess the same self-renewal properties as CSCs, and their number increases progressively in the p53 null premalignant mammary gland (156). However, according to Leonova *et al.*, although a small molecule inhibitor of p53 stimulates amplification of hematopoietic stem cells it does not promote tumor development in mice (157). Other studies report that pharmacological reactivation of p53 correlates with restoration of asymmetric divisions in CSCs and tumor growth reduction (156). These data suggest that loss of p53 favors symmetric divisions of stem cells, contributing to tumor growth. This notion was supported by the report of Paulson, who demonstrated that activation of p53 by MDM2 inhibitor reduced primary sphere formation, the characteristics of CSCs, in wild type p53-expressing MCF7 cells by 80% and completely eliminated secondary sphere forming cells (158). However, SUM159 and SUM149 cells with mutated p53 were unaffected by the MDM2 inhibitor which implied a p53-specific effect on sphere formation of mammary carcinoma cells. These findings contribute to the fact that targeting the CSC population through activation of p53 could be an effective therapy in patients with wild-type p53. Using mammary tumors arising spontaneously from transplants of BALB/c-*Trp53*$^{-/-}$ mammary epithelium, Zhang *et al.*, have shown that cells expressing markers of mouse mammary stem cells (lin^{-}/CD29^{hi}/CD24^{hi}) had a greater tumor-initiating frequency (159). The lin^{-}/CD29^{hi}/CD24^{hi} population shared additional features of mammary stem cells, including radiation resistance and the formation of secondary mammospheres (159). Using a unique culture model of luminal breast epithelial cells, Godar *et al.*, demonstrated that p53 binds to the promoter of *CD44*, a commonly used marker of CSCs, and represses CD44 expression (160). On the contrary, constitutive expression of CD44 blocks p53-dependent apoptosis and rendered cells resistant to doxorubicin (160). Indeed, apart from CD44, p53 represses the expression of more than 20 target genes that may contribute to maintenance of the pool of tumor-initiating cells (161). In addition, p53 has been shown to interact with numerous other proteins and signaling pathways that are involved with regulation of the CSC population (161). Loss of p53 would also allow increased expression of multidrug-resistance genes (ABCB1 or MDR1) that renders CSCs resistant to chemotherapies (161). Similarly both increased proliferation and decreased apoptosis of CSCs would be expected to result from de-repression of cdc25c and BIRC5/Survivin when p53 function is disrupted (162). NUMB is implicated to regulate the switch between self-renewal and differentiation in normal and cancerous cells through its target gene *Notch* (163). Colaluca *et al.*, showed that NUMB, p53 and MDM2 form a tri-complex that inhibits p53 degradation, resulting in increased p53-independent of Notch activity (163). The authors also suggest that deregulation of this interaction between NUMB and p53 could be one mechanism of tumorigenesis

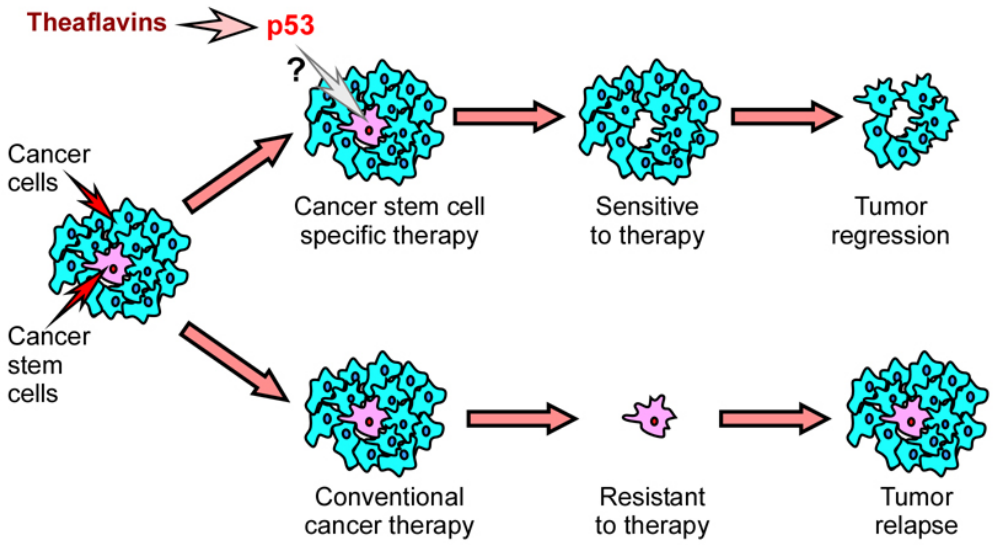

Figure 8. Theaflavins and cancer stem cells: Our hypothesis: A schematic diagram showing that cancer relapses due to the inability of conventional chemotherapies to induce apoptosis in cancer stem cells, the root cause of tumorigenesis. Since theaflavins efficiently regulate different aspects of tumorigenesis by targeting p53, we hypothesize that theaflavins may actually target CSC population via p53 to cause cancer regression.

due to a shift towards symmetric self renewal in the stem cell population. These findings indicate that targeting p53 therapeutically could have a more widespread effect on the 'root of all evils', i.e., CSC population, and related signaling pathways.

7. CAN THEAFLAVINS UP ROOT THE 'ROOT OF ALL EVILS' BY TARGETING P53? : A HYPOTHESIS

Through the revolutionized concept of CSCs, cancer research has been reinvigorated to study the role of these unique cells in cancer propagation and more importantly as targets in innovative therapies. On the basis of the above discussions on theaflavin-p53 cross-talk as well as on the relationship between p53-mutation and CSCs, we hypothesize (Figure 8) that theaflavins may up root the 'root of all evils' i.e., CSCs. Our discussion further elaborating that theaflavins regulate different aspects of tumorigenesis, i.e., cancer metabolism, angiogenesis, metastasis, apoptosis and drug-resistance *via* p53, supports our hypothesis since all these above-mentioned aspects of carcinogenesis are now-a-days claimed to be the contribution of CSCs. Further support to our hypothesis arises from the information that both theaflavins and p53 inhibit Wnt/beta-catenin, a major component of CSC self renewal pathway (144, 164). However, further work is required to settle down the yet unresolved debate on 'theaflavin-p53 cross talk in inhibition of CSC'.

8. CONCLUDING REMARKS

The almost unprecedented amount of research performed on p53 has equipped us with a stunning wealth of information. As illustrated in this review, one may have to approach p53 not as a simple switch that determines cell fate single-handedly, but its functions in altered conditions which in an intricate network of signals and molecular interactions regulate several aspects of oncogenesis. Although p53-based drug therapies have identified large numbers of putatively active compounds (peptide and non-peptide drugs), but in only a few cases has their precise mode of action been determined. More importantly the challenges that are associated with peptide stability, transport and toxicity in human clinical trials are yet to be completely addressed. At the same time clinical efficacy of p53-based gene therapy is often limited as not all cells will be injected with p53. Therefore, to overcome the limitations of p53 therapeutics, it is of utmost importance to exploit compounds like theaflavins that are essentially anti-carcinogenic but at the same time also non-toxic. Meanwhile more studies regarding the effect of theaflavins on tumor angiogenesis, metastasis and drug resistance, or as a whole on CSCs, are required to be conducted. Also for these compounds to exert maximum potency *in vivo*, the understanding of the absorption and metabolism of tea polyphenols is crucial. Moreover, since the bioavailability of black tea is low, it is of utmost importance to synthesize derivatives and/or compounds retaining the molecular framework of theaflavins while exhibiting enhanced bioavailability at the same time.

Time is short and the dreams far fetched. With this in mind, one needs to march ahead in the arena of scientific discoveries stampeding all difficulties that obstructs the way to finally reach to a cancer-free dawn.

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Acronyms and definitions: ASPP: apoptosis-stimulating protein of p53 , E3 ubiquitin ligase: a protein that covalently attaches a ubiquitin moiety to lysine residues of target proteins, marking them for proteasomal degradation , Li-Fraumeni: a hereditary syndrome, caused by a p53 germ line mutation, that results in cancer onset at a very early age , MDM2: murine double minute 2; also known as HDM2 in human , MDM4: MDM2-like p53-binding protein; also known as MDMX, HDM4, HDMX , Orthologs: genes in different species that have evolved from a common ancestral gene , Paralogs: genes within a genome that have evolved by gene duplication , p53C: DNA-binding core domain of p53 , SAXS: small-angle X-ray scattering , T-p53C: superstable mutant of p53 core domain containing the point mutations M133L, V203A, N239Y, and N268D , TAD: transactivation domain

Abbreviations: AP1: Activator protein-1; Apaf-1; apoptosis protease-activating factor-1; bFGF: basic fibroblast growth factor; BAI1: brain-specific angiogenesis inhibitor 1; Bax: Bcl-2-associated X protein; CPEB: cytoplasmic polyadenylation element-binding protein; CSC: cancer stem cell; COX-2: cyclooxygenase-2; EPHA2: ephrin receptor A2; EGFR: epidermal growth factor receptor; GST: Glutathione S-transferase; GLUT: glucose transporter; HIF: hypoxia inducible factor; HPV: human papilloma virus; iNOS: inducible nitric oxide synthase; JNK: c-Jun N-terminal kinase; MnSOD: manganese superoxide dismutase; MAPK: mitogen-activated protein kinase; MMP: matrix metalloproteinase; MVD: microvessel density; MDR: multidrug resistance; MDM2: murine double minute2; NFkB: nuclear factorkappa B; NCI: national cancer institute; POX: proline oxidase; Pin 1: prolyl isomerase; PI3K: phosphatidylinositol-3-kinase; PKC: protein kinase C; PAI-1: plasminogen activator inhibitor-1; PGM: phosphoglycero mutase; PUMA: p53-upregulated modulator of apoptosis; ROS: reactive oxygen species; SCO2: synthesis of cytochrome c oxidase 2;

STAT: signal transducer and activator of transcription; TGFbeta: Transforming growth factor beta; TSP-1: thrombospondin-1; TIGAR; TP53-induced glycolysis and apoptosis regulator; VEGF: Vascular endothelial growth factor.

Key Words: Angiogenesis, Apoptosis, CSC, Drug Resistance, Metastasis, p53, Theaflavins, Review

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