Using Boolean Logic Modeling of Gene Regulatory Networks to Exploit the Links Between Cancer and Metabolism for Therapeutic Purposes

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***Abstract*—The uncontrolled cell proliferation that is characteristically associated with cancer is usually accompanied by alterations in the genome and cell metabolism. Indeed, the phenomenon of cancer cells metabolizing glucose using a less efficient anaerobic process even in the presence of normal oxygen levels, termed the Warburg effect, is currently considered to be one of the hallmarks of cancer. Diabetes, much like cancer, is defined by significant metabolic changes. Recent epidemiological studies have shown that diabetes patients treated with the antidiabetic drug Metformin have significantly lowered risk of cancer as compared to patients treated with other antidiabetic drugs. We utilize a Boolean logic model of the pathways commonly mutated in cancer to not only investigate the efficacy of Metformin for cancer therapeutic purposes but also demonstrate how Metformin in concert with other cancer drugs could provide better and less toxic clinical outcomes as compared to using cancer drugs alone.**

***Index Terms*—Cancer, combination therapy design, diabetes, Metformin, Warburg effect.**

I. Introduction

CANCER has traditionally been described as a condition that evolves via a multistep process accumulating mutations with six essential genetic alterations or hallmarks leading to changes in cell physiology including genomic instability and increased mutability [1]. However, recently aerobic glycolysis or the "Warburg effect," where cancer cells switch to glycolysis (an event common in normal cells when there is a lack of oxygen) even when oxygen is available, which is an alteration in the metabolic phenotype, has been added as a seventh hallmark [2]. Type 2 diabetes mellitus (T2D) on the other hand has been described as a group of metabolic diseases in which a person has high blood sugar, either because the body does not produce enough insulin, or the cells do not respond to the insulin that is produced. There is thus a metabolic shift in T2D where abundant blood glucose, a key biological fuel essential for fast proliferating cancer cells, is available. Added to this scenario are the hyperinsulinemic [3] environment in T2D and increased gene expression of glycolytic enzymes in cancer [4], permitting an ideal microenvironment for tumor cells. Consequently, the connection of metabolic alterations in both diseases has juxtaposed these two conditions at the clinical, biological, and genetic levels [5]–[7].

Epidemiological studies have also demonstrated a positive association between T2D and the risk of cancer and cancer-related mortality [8]. Moreover, the diabetic drug Metformin has been shown to have a direct anticancer activity against breast and other cancers [9]. We exploit the association between T2D and cancer to develop a testable theoretical framework for cancer therapy design involving Metformin and chemotherapeutic drugs.

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II. Biological Background

Cancer cells exhibit unique metabolic characteristics. In this section, we discuss the features of tumor cell metabolism and look at how targeting altered tumor cell metabolism through Metformin might afford a therapeutic opportunity.

A. Cancer Cell Metabolism

Cancer cells are known to exhibit characteristic alterations in their metabolic activity [11]–[14]. Metabolism in normal cells differs in aerobic and anaerobic conditions. In the presence of oxygen, nonmalignant cells convert glucose to pyruvate through a multistep process called glycolysis. The pyruvate that is produced is transported to the mitochondria, the power house of the cell. The mitochondria then oxidize the pyruvate via a process called oxidative phosphorylation (OXPHOS) to generate adenosine triphosphate (ATP). ATP is the energy currency of the cell and is capable of storing large amounts of energy in its phosphoanhydride bonds. When the supply of oxygen is limited however, the cells shunt the pyruvate away from the mitochondria and convert it to lactate. Otto Warburg observed in the 1920s an anomalous characteristic of tumor cell metabolism that cancer cells even in the presence of oxygen opt for the latter route, i.e., irrespective of the extracellular levels of oxygen, cancer cells continue to metabolize glucose to lactate instead of utilizing mitochondrial OXPHOS. This peculiar characteristic

of cancer cell metabolism is called "aerobic glycolysis" or the "Warburg effect" [15].

On the face of it, the Warburg effect is counterintuitive as it is a highly inefficient method for energy production: for every molecule of glucose, glycolysis generates two molecules of ATP whereas OXPHOS produces 34 molecules. Cancer cells presumably have a high demand for energy so the metabolic switch to aerobic glycolysis does not seem rational. There are a number of reasons for this switch, the most important of which is that it allows cancer cells to divert the intermediate bimolecular products of the glycolytic chain toward biosynthetic pathways. Normal cells, in the quiescent state, produce energy as efficiently as possible. However, a cancer cell is tuned to incessant growth and proliferation. Toward this end, the tumor cells instead of metabolizing glucose with the goal of efficient energy generation divert the nutrients toward anabolic processes that will provide the necessary substrates for cell growth. Aerobic glycolysis allows cells to divert intermediates toward biomass accumulation. In order to make up for the inefficiency of aerobic glycolysis, the cancer cells take up much larger amounts of glucose. Indeed, enhanced glucose uptake and the accompanying increased glycolytic flux is a universal metabolic alteration in cancer, and forms the basis of the positron emission tomography scan technique for cancer detection. Another advantage of aerobic glycolysis is that it confers better survivability in an oxygen starved (hypoxic) environment, conditions which are common in tumor tissue [16].

One of the principal mechanisms behind the switch to aerobic glycolysis is the constitutive expression of the Hypoxia Inducible Factor 1 (HIF-1), which is a transcription factor ordinarily activated by hypoxic stress [14], [17]. HIF-1 drives many of the metabolic adaptations in cancer [13], [18]. First, it increases the uptake of glucose by upregulating the glucose transporters. Second, it increases glycolytic flux by activating enzymes in the glycolytic pathway. Third, it shunts pyruvate away from the mitochondria (pyruvate enters the tricarboxylic acid (TCA) cycle in the mitochondria through conversion to acetyl-CoA. This reaction is catalyzed by pyruvate dehydrogenase (PDH). HIF-1 inhibits PDH by activating PDH kinase 1 thereby slowing the entry of pyruvate into the TCA cycle). Finally, HIF-1 activates the enzyme lactate dehydrogenase A which catalyzes the conversion of pyruvate to lactate, and upregulates monocarboxolate transporter 4 to discharge the lactate into the extracellular matrix (ECM). This leads to the acidification of the ECM milieu which in turn promotes metastasis. Therefore, HIF initiates a transcriptional cascade which acts as a key driver of the metabolic adaptation of cancer cells.

In addition to the Warburg effect, another major facet of the metabolic reprogramming in cancer cells is increased "de novo fatty acid synthesis," a process where the cells synthesize the requisite lipids in-house rather than relying on the circulating exogenous supply from the blood stream as normal cells do [19]. The endogenous synthesis of lipids requires citrate which is derived from the TCA cycle. To sustain the TCA cycle, the depleted citrate is replenished through a process called anaplerosis [13]. Sterol regulatory element binding protein (SREBP) is a master transcriptional regulator of genes involved in de novo lipid and sterol biosynthesis [20]. Enhanced expression of SREBP has been shown to correlate with breast cancer progression [19].

Thus, fundamental alterations in tumor cell metabolism include aerobic glycolysis and de novo lipid synthesis with HIF-1 and SREBP as key markers of the metabolic reprogramming that takes place in cancer cells.

B. Metformin and Cancer

Altered tumor metabolism is of paramount importance for sustained uncontrolled cell growth, a vulnerability that can be exploited for therapeutic intervention [14], [19]. In the context of targeting cancer metabolism, the widely used antidiabetic drug Metformin has garnered attention for its potential anticancer properties suggesting a role for this drug in cancer therapy and prevention [21]–[24]. Tumor cells have a voracious appetite for glucose. Metformin suppresses hepatic gluconeogenesis which reduces glucose levels thereby diminishing the tumor fuel supply. The principal mechanism of action of Metformin is the activation of adenosine monophosphate-activated protein kinase (AMPK), the cellular energy sensor which when activated switches on ATP-generating pathways and diminishes energy-consuming biosynthetic processes thereby curtailing proliferation. Activation of AMPK by Metformin phosphorylates the tuberous sclerosis complex which in turn inhibits the mammalian target of rapamycin (mTOR) complex, the master stimulator of protein synthesis and cell growth.

In view of the central role of energy metabolism in cell proliferation, we investigate the therapeutic value of Metformin for cancer treatment. Moreover, there are several other potential benefits of adding Metformin to cancer therapy regimens. It is an FDA approved stable oral agent with a long history of use, is widely available, has an extremely low toxicity profile, and is very inexpensive [25].

III. Therapy Design

Cancer is an umbrella term for a set of diseases characterized by a break down in cell cycle control that allows cells to escape the usual controls on cell proliferation and survival. In [26], the authors take the view that in essence, it is a disease that results in aberrant signaling caused by breakdown(s) in the normal signaling pathway of a given cell, and therefore, it can be meaningfully treated or managed through remedying the effect of such breakdown(s). By adopting a similar approach, we consider the signaling pathways commonly mutated in cancer, map the biological pathway information to a digital circuit which is then used to determine the possible fault locations and devise an appropriate therapeutic scheme.

A. Pathway Model

Mutations in the PI3K/AKT/mTOR and Ras/MEK/ERK (MAPK) signaling pathways are common in breast cancer malignancies with frequent genetic alteration in several key players from these pathways [27], [28]. These pathways are activated via growth factor receptor tyrosine kinases and regulate cell metabolism, survival, and growth. A schematic representation

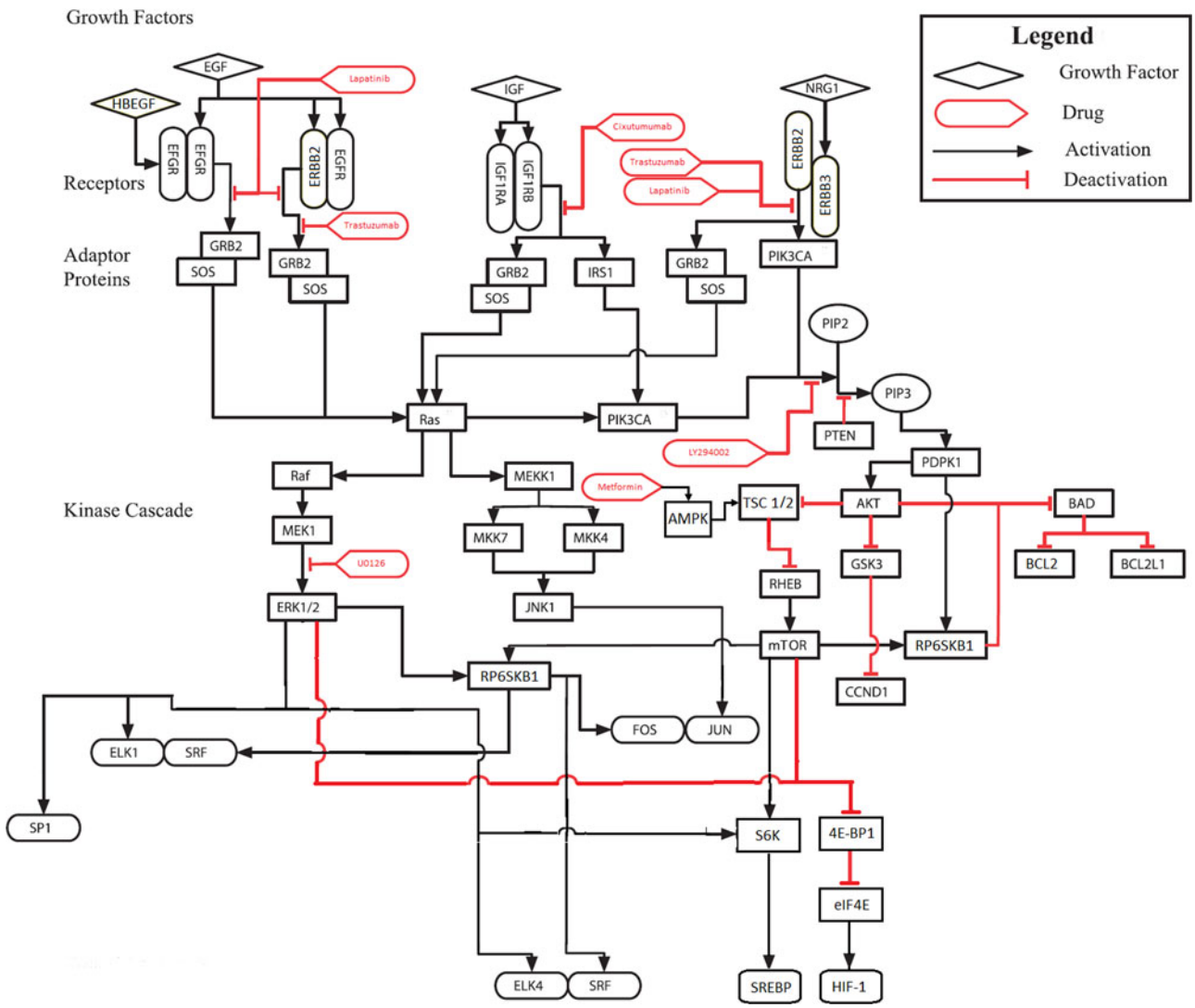

Fig. 1. Schematic diagram of pathways commonly mutated in breast cancer.

of these pathways is given in [26]. To this schematic, we add the pathway segment incorporating the transcription factors HIF-1 and SREBP. This leads us to our signaling pathway model of Fig. 1. The black and red lines in the diagram indicate relationships which are activating and inhibitory, respectively.

The red boxes show breast cancer drugs and their points of intervention in the pathway. The cancer drugs in our model are "targeted molecular therapies," agents which act with great specificity on particular molecules in the signal transduction network known to be important in cancer [29]–[32]. The cancer drugs are Lapatinib (a dual tyrosine kinase inhibitor of EGFR and ERBB2) [29]–[32], Trastuzumab (a monoclonal antibody targeting ERBB2) [29]–[32], Cixutumumab (anti-IGF1R monoclonal antibody) [33], U0126 (MAPK pathway inhibitor targeting MEK) [34], [35], and LY294002 (PI3K/Akt pathway inhibitor targeting PIK3CA) [30]–[32].

Genes exhibit switch-like on/off behavior and thus a gene regulatory network (GRN) can be modeled with a Boolean circuit [26]. The marginal interactions amongst genes represented by the signal transduction network can be translated to an equivalent Boolean network. For example, if either of two genes say A or B can activate a third gene C, then this component of the GRN can be represented by an OR gate with inputs A and B and output C. Using such a procedure outlined in [26] of translating the interactions of different genes in a signaling network to a logic circuit, we can model the pathways in Fig. 1 with a Boolean circuit, to arrive at Fig. 2. In this circuit, there are nine outputs, six of which are transcription factors (marked in yellow) and the remaining (which are not colored) reflect the activation status of some key proteins from our target signaling pathways.

B. Fault Locations and Drug Intervention Points

In normal cells, cell division is under extremely tight control and cells only divide to form further cells if they receive external signals to do so. These external signals that stimulate a cell to divide are called growth factors or mitogens. Cancer is characterized by a breakdown in cell signaling in which cells are set-free from the usual controls on cell-cycle progression

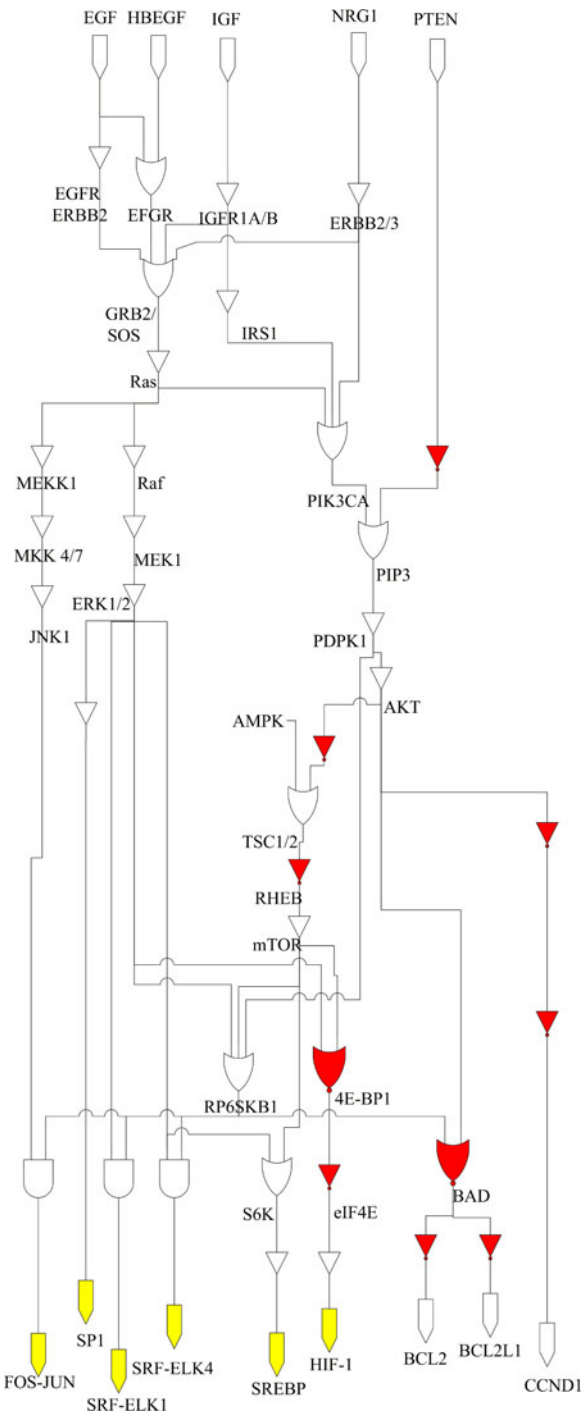

Fig. 2. Boolean circuit model.

and continue to grow and proliferate even in the absence of mitogenic signaling. Such abnormalities in the signaling network can be modeled as stuck-at faults where a point in the network is permanently fixed (stuck) at a particular value of either 1 (stuck-at-1 fault) or zero (stuck-at-0 fault) corresponding, respectively, to the constitutive (perpetual) activation or inactivation of a gene [26]. For example, in cancerous cells, the proto-oncogenes can get mutated to become oncogenes or a tumor suppressor gene can lose its braking function. For instance, if the PIK3CA proto-oncogene, a gene frequently mutated in breast cancer [27], mutates to PIK3CA oncogene, the encoded PIK3CA oncoprotein can become constitutively active and start perpetually signaling to the downstream proteins. In that case, even if there is no mitogenic signaling from the outside, the cell will be stimulated to divide. Such constitutive activation of PIK3CA can be modeled as a stuck-at-1 fault. Similarly, mutation in the PTEN tumor suppressor can cause a cell to ultimately undergo uncontrolled cell division, and possibly turn cancerous. Such a fault that renders PTEN inactive corresponds to a stuck-at-0 fault. Thus, cancer is a disease of aberrant cell signaling caused by failures in the signaling pathways which can be represented as stuck-at faults in the network. For simplicity, we consider single stuck-at faults. From the network in Fig. 2, we identify 27 possible fault locations illustrated in Fig. 3 with the "stuck-at-1" faults in black numerals and the "stuck-at-0" faults in red.

The intervention points in the Boolean circuit for the cancer drugs are shown in Fig. 4. Since the cancer drugs of Fig. 1, with the exception of Metformin, break or stop the effect of the kinase (to which they bind) on the molecules further downstream of the signaling cascade, a drug of this type can be modeled as an inverted input to an "AND" gate at the point of intervention [26]. As discussed in Section II, Metformin however acts as an activator of the AMPK, overcoming the dysregulation of AMPK in cancer. Thus, in order to incorporate the effect of Metformin in our Boolean network, its action is modeled via the activation of AMPK.

C. Fault Classification

In this section, we group the faults identified in Fig. 3 into different classes of equivalent faults based on their output to the nonproliferative input. The input and output vectors are defined as follows:

$$\begin{aligned} INPUT = \ & V = [EGF, HBEGF, IGF, NRG1, PTEN] \\ OUTPUT = \ & [FOS\text{-}JUN, SP1, SRF\text{-}ELK1, \\ & SRF\text{-}ELK4, SREBP, HIF\text{-}1, \\ & BCL2, BCL2L1, CCND1]. \end{aligned}$$

Each input can take on binary values. We set the input vector to [00001] which corresponds to the growth factors being absent and the tumor suppressor PTEN being active, i.e., nonproliferation. For each of the 27 faults that may cause cancer, the output is tabulated in Fig. 5(a). Fault location zero corresponds to the fault-free case.

Based on the outputs, the faults can be grouped together into classes of equivalent faults. Faults which produce identical output for the same input test vector are equivalent. From the outputs in Fig. 5(a), the sets of equivalent faults for the test vector $V = [00001]$ are shown in Fig. 5(b).

D. Simulation Results for Drug Intervention

The total number of drugs is 6, so we define a binary drug vector of length 6 with each component having a value of either 1 if the corresponding drug is applied, and zero if it is not.

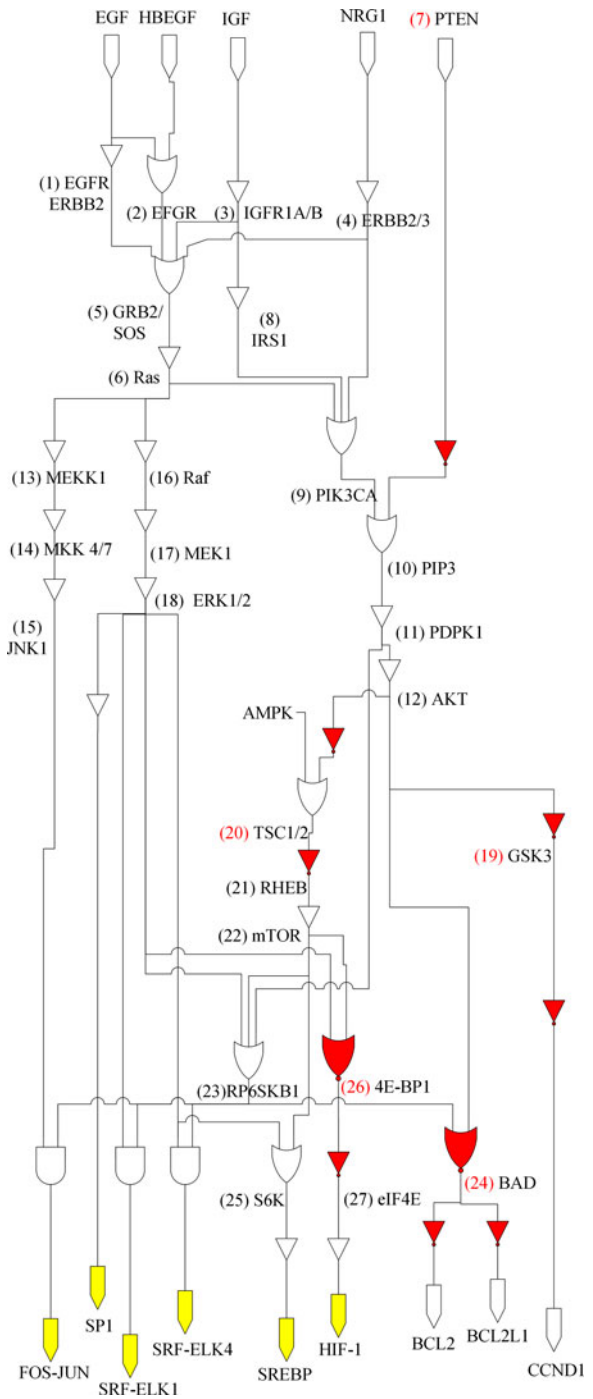

Fig. 3. Possible fault locations.

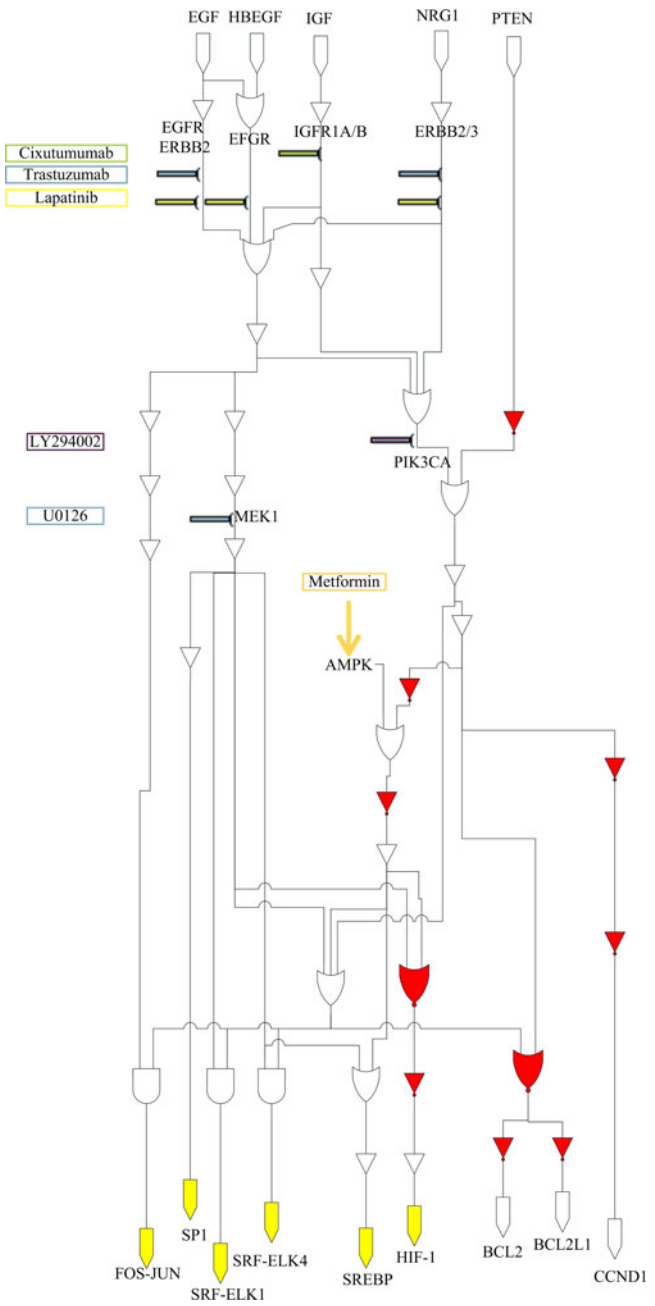

Fig. 4. Drug intervention locations.

Thus, there are a total number of $2^6 = 64$ possible drug vectors representing all the possible drug combinations. For each of the 27 faults that may cause cancer, the simulation determines the output for every drug combination and maps the output to a real number indicating the extent of proliferation. The drug vector is defined as follows:

$$DRUG\ VECTOR = [Metformin, Lapatinib, Trastuzumab, Cixutumumab, U0126, LY294002].$$

The input and output vectors are defined as before in the previous section. Again, for the simulation, we set the input vector to [00001], i.e., a nonproliferative input. For this input, we expect all the outputs comprising proliferative transcription factors, metabolic adaptation markers, cell-cycle progression and antiapoptotic proteins to be deactivated or turned off and indeed, for the fault-free scenario, this is certainly the case. However, faults in the signaling network can cause a proliferative (nonzero) output even for the nonproliferative input. Our objective is to nullify the effect of the faults by targeted drug intervention to produce an output as close to [000000000] and as far away from the extremely proliferative output [111111111] as possible.

Fault Location	Output								
	FOS-JUN	SP1	SRF-ELK1	SRF-ELK4	SREBP	HIF-1	BCL-2	BCL2L1	CCND1
0	0	0	0	0	0	0	0	0	0
1	1	1	1	1	1	1	1	1	1
2	1	1	1	1	1	1	1	1	1
3	1	1	1	1	1	1	1	1	1
4	1	1	1	1	1	1	1	1	1
5	1	1	1	1	1	1	1	1	1
6	1	1	1	1	1	1	1	1	1
7	0	0	0	0	1	1	1	1	1
8	0	0	0	0	1	1	1	1	1
9	0	0	0	0	1	1	1	1	1
10	0	0	0	0	1	1	1	1	1
11	0	0	0	0	1	1	1	1	1
12	0	0	0	0	1	1	1	1	1
13	0	0	0	0	0	0	0	0	0
14	0	0	0	0	0	0	0	0	0
15	0	0	0	0	0	0	0	0	0
16	0	1	1	1	1	1	1	1	0
17	0	1	1	1	1	1	1	1	0
18	0	1	1	1	1	1	1	1	0
19	0	0	0	0	0	0	0	0	1
20	0	0	0	0	1	1	1	1	0
21	0	0	0	0	1	1	1	1	0
22	0	0	0	0	1	1	1	1	0
23	0	0	0	0	0	0	1	1	0
24	0	0	0	0	0	0	1	1	0
25	0	0	0	0	1	0	0	0	0
26	0	0	0	0	0	1	0	0	0
27	0	0	0	0	0	1	0	0	0

(a)

Output	Equivalent Fault Groups
000000000	0,13,14,15
111111111	1,2,3,4,5,6
000011111	7,8,9,10,11,12
011111110	16,17,18
000000001	19
000011110	20,21,22
000000110	23,24
000010000	25
000001000	26,27

(b)

Fig. 5. (a) Output vector for all single stuck-at faults with input $V = [00001]$. (b) Equivalent fault groups for input $V = [00001]$.

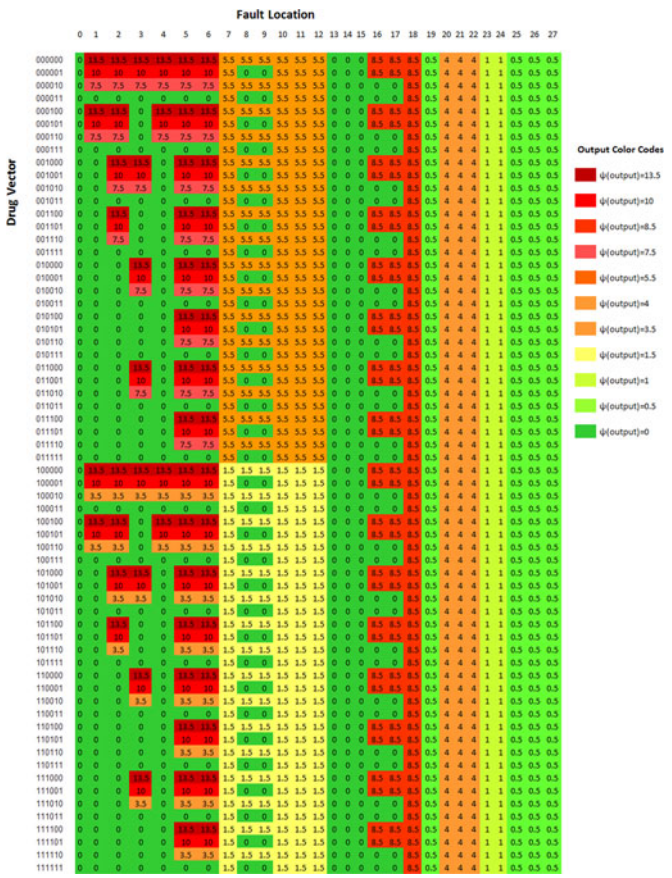

Fig. 6. Drug vector response.

To quantify the degree of abnormal behavior, we define a transformation as in [26] to map the $2^9 = 512$ output vectors to the continuous real number scale. Since the first six components of the output vector are transcription factors, and the remaining are the activation status of some proteins, these two groups of outputs have different biological significance and are encoded separately. Defining N_1 to be the number of active transcription factors and N_2 to be the number of active remaining outputs, the transformation below maps in a many-to-one fashion the output vector to a real number:

$$\begin{aligned} Output &= [a, b, c, d, e, f, g, h, i] \\ N_1 &= [a + b + c + d + e + f] \\ N_2 &= [g + h + i] \\ P &= N_1 \times N_2 \\ S &= N_1 + N_2 \\ \psi(output) &= \alpha P + (1 - \alpha) S \end{aligned}$$

where $\alpha \varepsilon (0, 1)$ is a free design parameter that defines the convex combination of the sum and product of N_1 and N_2. It determines the relative weights assigned to the sum and product. Since there is no obvious reason to assign greater weight to the sum or product term relative to the other, we assign equal weights by selecting a value for α that is right in the middle of the parameter space. Hence, α is chosen to be 0.5 for the simulation. Thus, we have $\Psi = 0.5(N_1 N_2 + N_1 + N_2)$. Note that this is a nonlinear cost function with a product term $N_1 N_2$. As a consequence, this transformation produces a higher cost (for the same number of total active output components), when genes from both groups in the output vector are expressed compared to the situation when only one group in isolation is trying to drive proliferation. This makes sense as we expect that the extent of proliferation will be higher when both sets of genes, which when deregulated play important complementary roles toward unchecked proliferation, are simultaneously active. It is pertinent to point out, however, that the results that follow are not predicated on the exact definition of the mapping.

The function Ψ's values for all possible faults and drug combinations are shown in Fig. 6 with the fault locations and drug vectors along the horizontal and vertical directions, respectively. The outputs are color-coded on a scale with red representing extreme proliferation and green nonproliferation. The color codes used are listed on the right side of Fig. 6. Again fault location zero corresponds to the fault-free case.

We would like to drive as many of the faults toward green (nonproliferation) as possible using as few of the cancer drugs as

we can since these drugs have toxic side effects. From Fig. 6, we can immediately see the benefit of Metformin. It mitigates the effect of faults 7–12, which are faults in the insulin/insulin-like growth factor (IGF) signaling pathway and the PI3K/AKT pathway, the pathway involved in regulating cell metabolism. From the previous section, these faults are classified in the same fault group and it is for this class of faults that we expect Metformin to show therapeutic benefit, i.e., Metformin should ameliorate the effect of faults that lead to the deregulation or hyperactivation of the PI3K/AKT/mTOR axis. Indeed, Metformin has been shown to overcome the dysregulation of the PI3K pathway by suppression of mTOR through AMPK activation in breast cancer cells. A number of studies have indicated the antitumorigenic effects of Metformin in multiple cancer cell lines including breast cancer with the use of Metformin as an anticancer agent now being evaluated in clinical trials [21], [24], [36]–[38]. Moreover, one of the most common ways in which the PI3K/AKT/mTOR pathway can be deregulated in breast cancer is the loss of PTEN, the negative regulator of this cascade, an event found in up to 40% of breast tumors [39]. Metformin has been shown to delay the onset of tumors in PTEN-negative mice [40]. Experimental studies have also demonstrated the benefit of Metformin in combination with chemotherapeutic agents and provided a rationale for Metformin as part of combination therapy for breast cancer [41]. All in all, our simulation results with respect to the therapeutic benefit of Metformin for cancer seem to be in concordance with the literature.

Note that no drug vector has any effect on fault 18. This makes sense as fault 18 corresponds to a mutation in the ERK1/ERK2 protein. This fault is downstream of all the drugs in our pathway model, so no drug combination is able to counteract the effect of this particular fault. In addition, the faults 13–15 produce an all zero output, i.e., these faults are "undetectable" as they generate the same output as the fault-free case. Since we are only concerned with faults that can induce cancer, we do not have to worry about these particular faults as they produce a nonproliferative output.

The best two-drug vector in terms of driving faults toward green is 100010, the combination of Metformin and U0126. However, considering cancer drugs only (i.e., excluding Metformin) the best drug vector is 000011, i.e., the drug combination of U0126 and LY294002 is the best two drug combination of cancer drugs. If to this combination we add Metformin, we see we get an even better result as more of the faults are driven toward green. This better outcome is obtained at minimal additional cost as in contrast to cancer drugs, Metformin is inexpensive and does not have adverse side-effects. We conclude that incorporating Metformin in the mix for cancer therapy can lead to improved outcomes.

Therefore, it seems that U0126 and LY294002 along with Metformin should be a potent combination therapy for breast cancer. We thus propose that a cancer combination therapy of U0126 or some other MEK (mitogen-activated protein kinase) inhibitor and LY294002 along with Metformin can lead to better therapeutic results. The exact same cancer drug combination of U0126 and LY294002 has been proposed as a therapeutic approach in the prevention and treatment of human melanoma [42]. Furthermore, recently a similar drug combination that targets the MAPK and PI3K pathways has shown promising results for Rhabdomyosarcoma [43]. Since these very signaling pathways are the most frequently deregulated signaling cascades in human breast cancer [27] [28], it is reasonable to expect the previous drug combination to be effective for breast cancer malignancies. The fact that our model prediction regarding the therapeutic potential of Metformin is in consonance with the literature and that the proposed cancer drug combination has shown promising results in other cancers with frequent mutations in the same pathways suggests that this particular cancer therapeutic regimen warrants further investigation. In any case, our results indicate at a minimum that incorporating the metabolism-targeting drug Metformin in the cancer therapy cocktail should give better outcomes compared with the use of cancer drugs alone.

Thus, by computer simulation of the Boolean logic equivalent model of the critical gene regulatory pathways of breast cancer, we have been able to demonstrate the benefit of Metformin use in cancer therapy which suggests a role for this drug in combination with cancer drugs. The theoretical results presented herein regarding the potential benefits of including the metabolism targeting drug Metformin as part of a combination cocktail therapy for cancer ultimately need to be validated via actual experiments on cancer cell lines.

The work presented here has some limitations. One of the major impediments to the success of therapeutic intervention in cancer is the presence of feedback signaling. Any attempt to counter the deregulation of a particular signaling pathway by administering targeted therapy is counteracted in cancer by exploiting the redundancy in the cellular signaling network through the compensatory activation of feedback loops which ultimately limit the potency of any attempted therapeutic intervention [44]. Furthermore, a tumor population is generally heterogeneous in that it is comprised of a number of different subpopulations that harbor distinct mutations with the result that no two cancers are completely alike [45]. As a consequence, different subpopulations require different treatments. These issues of tumor heterogeneity and feedback signaling are complex research problems in their own right but need to be addressed, i.e., a comprehensive system model needs to incorporate these considerations. Some preliminary progress in this direction has been made [46], [47].

IV. Conclusion

Cancer cells are known to show atypical metabolic characteristics: an "Achilles' heel" [13] that provides a therapeutic opportunity. We have investigated via simulations the benefit of targeting tumor cell metabolism by using the antidiabetic drug Metformin. The biological pathways involved in cell growth and metabolic regulation were mapped to a Boolean network. The equivalent digital circuit was used to identify locations at which faults could occur and categorize them into equivalent classes based on their output. We incorporated the drug intervention points into our model allowing us to test different combination therapies in terms of their efficacy in mitigating the effects

of faults in the network. We have shown that incorporating Metformin in the therapeutic regimen can lead to better outcomes. We predict that a combination therapy of Metformin and cancer drugs will lead to improved cancer therapy design. One of our long term objectives is to experimentally validate such predictions using cancer cell lines.

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