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A SYSTEM MODEL FOR CELL DEATH/ SURVIVAL USING SPICE AND LADDER LOGIC

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Systems Biology is the science of discovering, modeling, understanding and ultimately engineering at the molecular level the dynamic relationships between the biological molecules that define living organisms. Computational modeling is useful as a means to assemble and test what we know about proteins and networks. Models can help address key questions about the measurement, definition and function. Computationally intensive simulations can incorporate greater numbers of neurons to model increasingly complex and realistic properties, both electrical and chemical. Recent studies suggest that the balance between cell survival signals and pro-apoptotic stimuli controls the decision between cell repair and death. Inspired by the computational feasibility of Simulation Program For Integrated Circuit Emphasis (SPICE), and Ladder logic we set out to build a systematic signaling network that would enable the predictive signal of cell death/ survival given Tumor necrosis factor-α (TNF), Epidermal growth factor (EGF) and Insulin as inputs. We conclude that it is possible to build self consistent prediction model that can be computationally to yield important insights into the control of cell death/ survival responses.

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Keywords: Tumor necrosis factor-α, Epidermal growth factor, Insulin, SPICE, Ladder logic

I. Introduction

Cell signaling pathways interact with one another to form networks. Such networks are complex in their organization and exhibit emergent properties such as bistability and ultrasensitivity [1]. Analysis of signaling networks requires a combination of experimental and theoretical approaches including the development and analysis of models. This work examines signaling networks that control the survival decision treated with combinations of three primary signals [2, 3]; the pro death cytokine, *tumor necrosis factor-α* (TNF), and the pro survival growth factors, *epidermal growth factor* (EGF) and insulin. TNF activates intracellular signals by binding to trimeric death receptors and promoting assembly of intracellular death-inducing signaling complexes (DISCs) [4, 5, 6]. Activation of the EGF receptor tyrosine kinase (EGFR) [7, 8] occurs through receptor dimerization, conformational change, and auto phosphorylation [9, 10]. Phosphorylated receptors recruit adaptor proteins, and these then activate multiple signaling proteins including extracellular-regulated kinase (ERK) via Ras [11] and the Akt [12] kinase via phosphatidylinositol 3- kinase (PI3K).The binding of insulin to the insulin receptor also activates ERK and Akt, but in contrast to EGFR, the insulin receptor is constitutively dimerized, and most insulin induced signaling involves modification of insulin receptor substrate 1 (IRS1) [13, 14], a multi domain adaptor protein.

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A Programmable Logic Controller (PLC) has many "input" terminals, through which it interprets "high" and "low" logical states from sensors and switches. It also has many output terminals, through which it outputs "high" and "low" signals to power lights, solenoids, contactors, small motors, and other devices lending themselves to on/off control. In an effort to make PLCs easy to program, their programming language was designed to resemble ladder logic diagrams. Thus, an industrial electrician or electrical engineer accustomed to reading ladder logic schematics would feel comfortable programming a PLC to perform the same control functions.

Ladder logic is a programming language that represents a program by a graphical diagram based on the circuit diagram. It is primarily used to develop software for Programmable Logic Controllers (PLCs) used in industrial control applications. The name is based on the observation that programs in this language resemble ladders, with two vertical rails and a series of horizontal rungs between them.Ladder notation is best suited to control problems where only binary variables are required and where interlocking and sequencing of binary is the primary control problem.

2. Computational model

The prediction model for cell death/survival has been implemented using SPICE. We have implemented the signaling system heading by three input signals such as TNF, EGF and insulin. The block diagram of the signaling system that was modeled is shown in Figure 1.

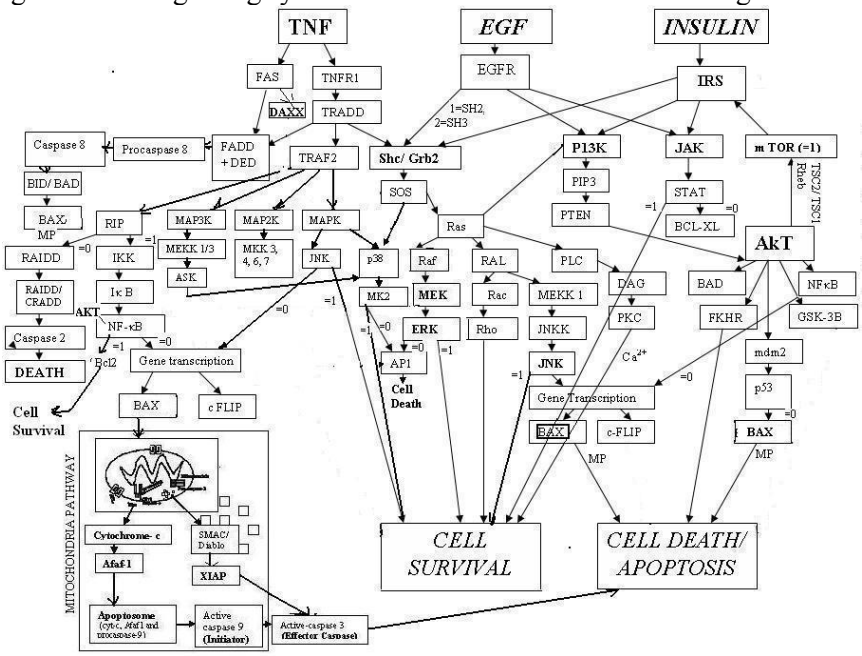


Fig. 1 Computational Model for cell survival/ death.

2.1 Tumor Necrosis Factor-α (TNF)

There are two receptors, **TNF-R1** (TNF receptor type 1) and **TNF-R2** (TNF receptor type 2), bind to TNF [4]. TNF-R1 is constitutively expressed in most tissues, and can be fully activated by both the membrane-bound and soluble trimeric forms of TNF, while TNF-R2 is only found in cells of the immune system and respond to the membrane-bound form of the TNF homotrimer. As most information regarding TNF signaling is derived from TNF-R1, the role of TNF-R2 is likely underestimated. Upon contact with their ligand, TNF receptors also form trimers, their tips fitting into the grooves formed between TNF monomers. This binding causes a conformational change to occur in the receptor, leading to the dissociation of the inhibitory protein SODD from the intracellular death domain. This dissociation enables the adaptor protein TRADD to bind to the

death domain, serving as a platform for subsequent protein binding. TNF receptor associated factor 2 (TRAF2) is a prototypical member of the TRAF family proteins that regulates signals from the TNF receptors, resulting in sequential activation of MAP3K (MEKK1/3, ASK1/2) [15], MAP2K (MKK3, 4, 6, 7) [16] and MAPK (JNK, p38) [17], as well as in activation of RIP/IKK signaling pathways. MAPK and IKK in turn activate AP-1 and NF-κB transcription factors. Activation of AP-1 and NF-κB induces genes involved in inflammation, immune response, cell proliferation and cell differentiation, as well as genes that act to suppress death receptor- and stress-induced apoptosis. The signaling pathways from RIP/IKK to NF-κB and from MAP3K to AP-1 are better understood, the receptor proximal events that determine TRAF2-dependent activation of RIP/IKK vs. MAP3K remain largely elusive shown in Figure 1.

Induction of death signaling: Like all death-domain containing members of the TNFR superfamily, TNF-R1 is involved in death signaling. However, TNF-induced cell death plays only a minor role compared to its overwhelming functions in the inflammatory process. Its death inducing capability is weak compared to other family members (such as Fas), and often masked by the anti-apoptotic effects of NF-κB. Nevertheless, TRADD binds FADD, which then recruits the cysteine protease caspase 8. A high concentration of caspase 8 induces its autoproteolytic activation and subsequent cleaving of effector caspases, leading to cell apoptosis [6, 18]. Cell death is an essential strategy for the control of the dynamic balance in living systems, and two fundamentally different forms of cell death, ***apoptosis and necrosis*** [19], have been defined.

2.2 Epidermal Growth Factor (EGF)

Upon ligand-binding receptors homo-dimerise or hetero-dimerise triggering tyrosine [20] trans-phosphorylation of the receptor sub-units. Intracellular tyrosine kinases of the Src family and Abl family are also able to tyrosine phosphorylate ErbB receptors. These tyrosine phosphorylated sites allow proteins to bind through their Src homology 2 (SH2) domains leading to the activation of downstream signaling cascades including the RAS/extracellular signal regulated kinase (ERK) pathway, the phosphatidylinositol 3 kinase(PI3K) pathway and the Janus kinase/Signal transducer and activator of transcription (JAK/ STAT) pathway. Differences in the C-terminal domains of the ErbB receptors govern the exact second messenger cascades that are elicited conferring signaling specificity. The EGF signal is terminated primarily through endocytosis of the receptor-ligand complex. A number of signal transduction pathways branch out from the receptor signalling complex as shown in Figure 1.

EGF activates the ERK pathway through the binding of Grb2 or Shc to phosphorylated ErbB receptors, which in turn results in the recruitment of the son of sevenless (SOS) to the activated receptor dimmer SOS then activates RAS leading to the activation of RAF 1[21]. RAF-1 subsequently phosphorylates MEK1 and MEK2 which activate respectively ERK 1 and ERK2.

MAP kinases are actually a family of protein kinases that are widely distributed and are are found in all eukaryotic organisms. These can be classified into three main functional groups [15, 16]. The ERK pathway responds to mitogen activation. In the JNK/SAPK pathway SAPK stands for stress activation protein kinase and within this class of kinases the Jun N-terminal kinases (JNK) for a subfamily. In the p38/HOG pathway HOG stands for high osmolarity glycerol where the p38 proteins are a subfamily. EGF also promotes cell survival through the activation of PI3 kinase/AkT signaling [1, 2, 3]. EGF triggers the recruitment of PI3 kinase to activated ErbB receptors, which is mediated by the binding of SH2 domains in PI3-kinase to phosphorylated tyrosine residues. PI3-kinase can also activate RAS, resulting in the activation of ERK signaling, thereby facilitating cross-talk between survival pathways. A key downstream effector of PtdIns(3,4,5)P_3 is AKT(PKB). AKT promotes cell survival through the transcription of anti-apoptotic proteins [12]. Intermediate transcription factors involved in this process are NFκB and CREB. Another downstream target of AKT is glucogen synthase kinase 3 (GSK3). Under basal conditions the constitutive activity of GSK3 leads to the phosphorylation and inhibition of a guanine nucleotide exchange factor eIF2B, which regulates the initiation of protein translation. AKT also activates mammalian target of rapamycin (mTOR) [22], which promotes protein synthesis through p70 ribosomal S6 kinase (p70s6k) and inhibition of eIF-4E binding protein (4E-BP1). Another signaling cascade initiated by EGF is the JAK/STAT pathway, which is also

implicated in cell survival responses [23]. JAK phosphorylates STAT proteins localized at the plasma membrane. This leads to the translocation of STAT proteins to the nucleus where they activate the transcription of genes associated with cell survival

2.3 Insulin

Activation of PI3K by insulin, insulin-like growth factor-1 resulted in a regulation of broad range of cellular functions. Akt (protein kinase B, c-Akt) is one of the serine/threonine kinases downstream of PI3K. Akt was originally implicated in cancer development, promoting cell proliferation and inhibition of apoptosis. Insulin and other growth factors acutely activate Akt**.** Once active, AKT enters the cytoplasm where it leads to the phosphorylation and inactivation of glycogen synthase kinase 3 (GSK3) leading to cell death.

Cell signaling mediated by G protein switch involves the tuberous sclerosis complex (TSC), tumor suppressors (TSC1 and TSC2) and the Ras-related small G protein Rheb. A complex between TSC1 and TSC2 is regulated by multi-site phosphorylation and acts as a point of integration for a diverse array of cellular signals, including those arising from growth factors, nutrients, and a variety of stress conditions. When active, the TSC1-TSC2 complex [24, 25] acts as a GTPase activating protein (GAP) for Rheb, thereby turning Rheb off by stimulating its intrinsic GTPase activity. In the presence of growth factors and nutrients, this complex is turned off, allowing the GTP-bound active version of Rheb to accumulate and turn on downstream pathways. The best-characterized downstream effectors of Rheb is the mammalian target of rapamycin complex 1 (mTORC1), a critical regulator of cell growth and proliferation.

3. Results and discussion

When studying logic, one must begin with the basic functions. Input values can be combined using the logical AND, OR, NOT functions. Logic gates use digital electronics to implement these functions. Each gate is actually a circuit, typically consisting of transistors and biasing resistors. As an example, the transistor-transistor logic (TTL) 7408 chip contains four, two-input AND gates in one integrated circuit (IC) package. These gates and other types on separate ICs can be wired together to implement a wide array of digital logic.

In case of ladder logic, logic functions are implemented by developing a ladder diagram. Named for its resemblance to a ladder, the diagram consists of two vertical rails connected by several horizontal rungs. Each rail is energized at a different voltage, and each rung contains at least one element, such as a relay coil or an indicator lamp, across which voltage can drop. In a ladder circuit, normally open (type-A) and normally closed (type-B) contacts are interconnected so as to implement the logical functions. Connecting two contacts in series implements an AND function, since the first and second contacts must both be closed to complete the circuit. Connecting the same two contacts in parallel implements a logical OR, since at least one contact must be closed to complete the circuit. The equation can also be manipulated to other forms that are more routine but less efficient. The equation shown is in disjunctive normal form- in simpler words this is ANDed (shown in Fig 2 (a)) terms ORed (shown in Fig 2 (b)) together. This is also an example of a canonical form - in simpler terms this means a standard form. This form is more important for digital logic, but it can also make some PLC programming issues easier. For example, when an equation is simplified, it may not look like the original design intention, and therefore becomes harder to rework without starting from the beginning.

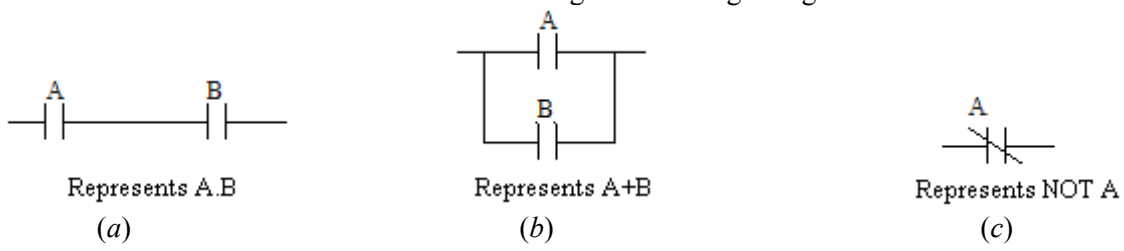


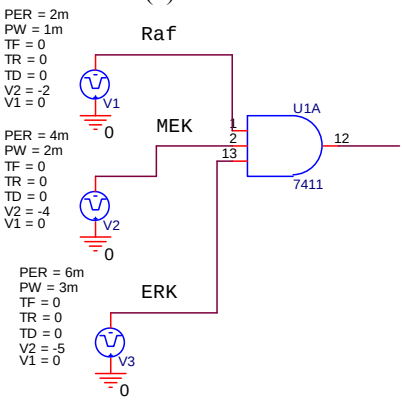
Fig 2. (a) Represents AND gate; (b) Represents OR gate; (c) Represents NOT gate

Above we had studied relating how TNF, EGF and Insulin work and its pathways in detail and explain each and every possible path for that. Based on pathways we had made truth tables for every possible path shown in Fig 3 (*a*), 4 (*a*), 5 (*a*), 6 (*a*) and 7 (*a*) for cell survival/death. Than we realize the truth tables by Karnaugh Map (K-Map) and get the Boolean expression for its individual possible paths. We simulate the results of each path, then combine all the results, and simulate through SPICE simulator using universal gates i.e AND, OR and NOT gate shown in Fig 3 (*b*), 4 (*b*), 5 (*b*), 6 (*b*) and 7 (*b*), get result for combination of TNF, EGF and Insulin for its cell survival/death. In output, '1' signifies cell survival and '0' signifies cell death shown in Fig 3 (*c*), 4 (*c*), 5 (*c*), 6 (*c*) and 7 (*c*). For cell survival the ten different proteins i.e. P13K, TNFR1, EGFR, IRS, IKK, Grb2, SOS, Ras, TRADD, Traf2 should present. If any one of them is absent than it will lead to cell death.

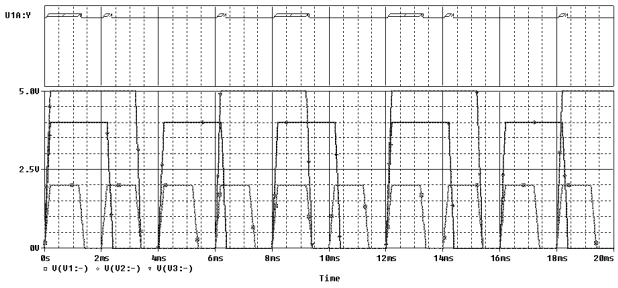
Next we have realized the Boolean equation using Ladder Logic shown in Fig 3 (*d*), 4 (*d*), 5 (*d*), 6 (*d*) and 7 (*d*).

RAF	MEK	ERK	Output
0	0	0	0
0	0	1	0
0	1	0	0
0	1	1	0
1	0	0	0
1	0	1	0
1	1	0	0
1	1	1	1

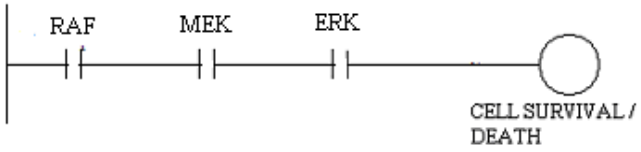
(*a*) Truth Table



(*b*) Boolean Circuit using Gates



(*c*) Output of Boolean circuit

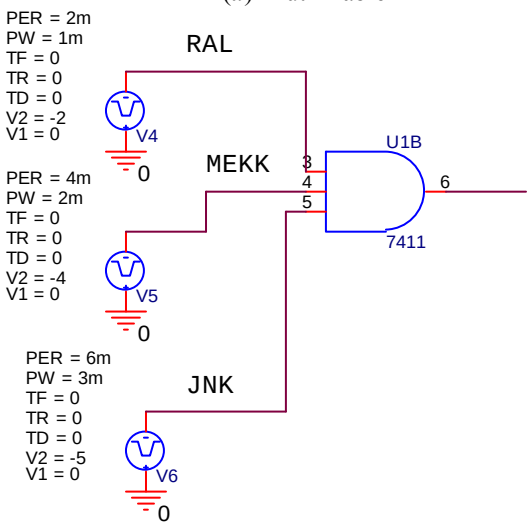


(*d*) Ladder Logic Circuit

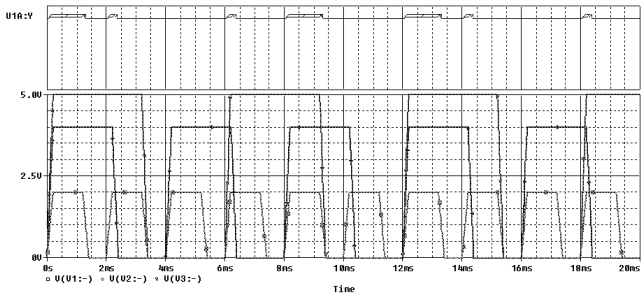
Fig 3 : Showing truth table, Boolean circuit, output for that circuit diagram, and Ladder logic diagram for RAF, MEK and ERK

RAL	MEKK	JNK	Output
0	0	0	0
0	0	1	0
0	1	0	0
0	1	1	0
1	0	0	0
1	0	1	0
1	1	0	0
1	1	1	1

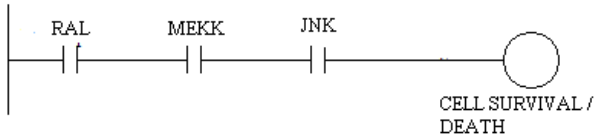
(*a*) Truth Table



(*b*) Boolean Circuit using Gates



(*c*) Output of Boolean circuit

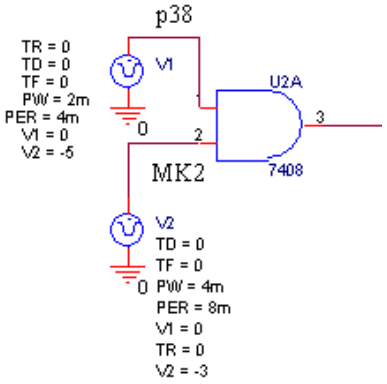


(*d*) Ladder Logic Circuit

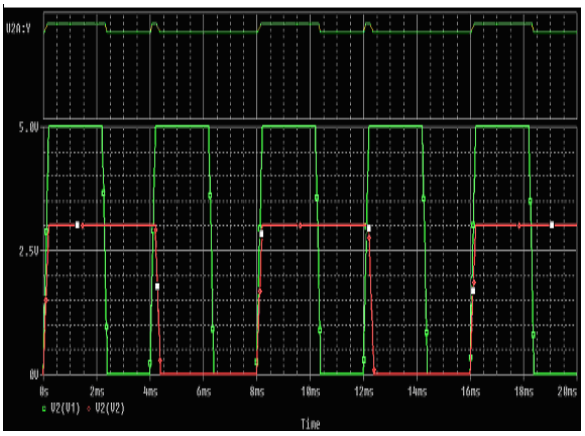
Fig 4. Showing truth table, Boolean circuit, output for that circuit diagram, and Ladder logic diagram for RALL, MEKK and JNK

p 38	MK2	Output
0	0	0
0	1	0
1	0	0
1	1	1

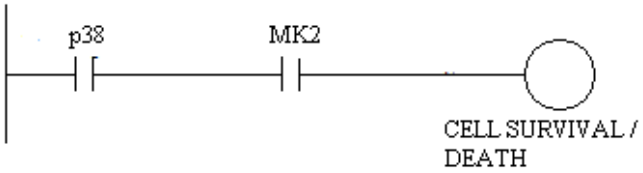
(*a*) Truth Table



(*b*) Boolean Circuit using Gates



(*c*) Output of Boolean circuit

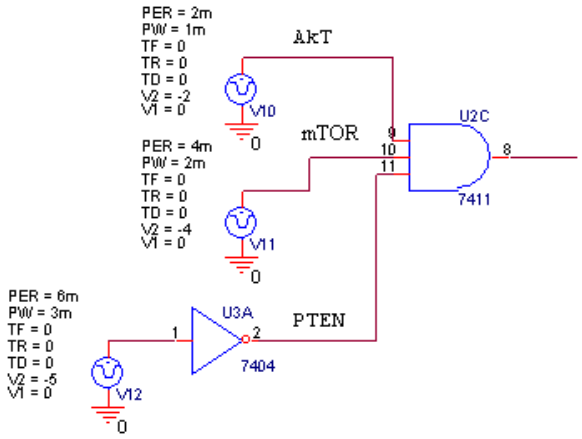


(*d*) Ladder Logic Circuit

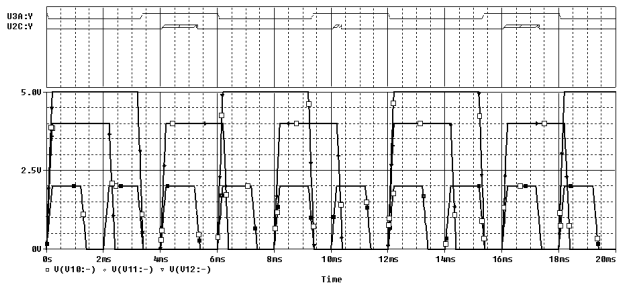
Fig 5. Showing truth table, Boolean circuit, output for that circuit diagram, and Ladder logic diagram for p38 and MK2

mTOR	PTEN	AkT	Output
0	0	0	0
0	0	1	0
0	1	0	0
0	1	1	0
1	0	0	0
1	0	1	1
1	1	0	0
1	1	1	0

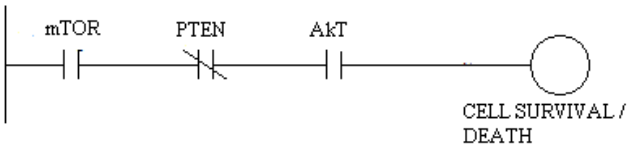
(*a*) Truth Table



(*b*) Boolean Circuit using Gates



(*c*) Output of Boolean circuit

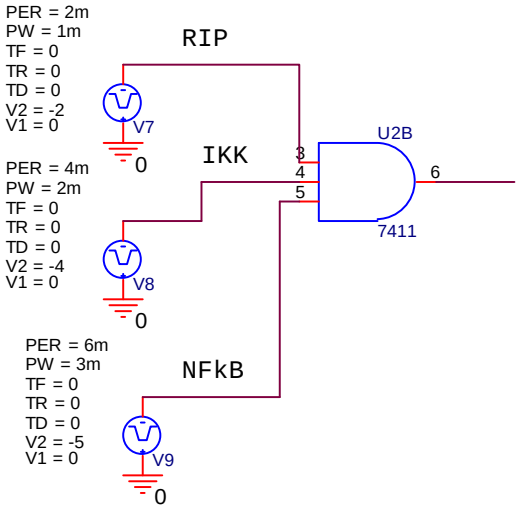


(*d*) Ladder Logic Circuit

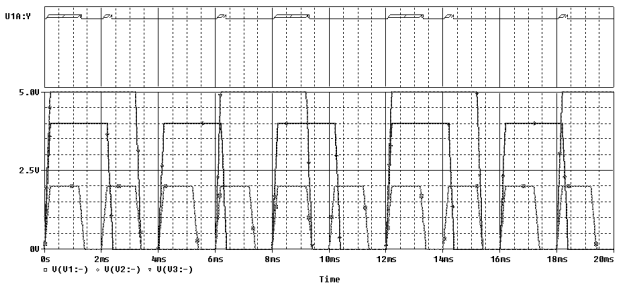
Fig 6 . Showing truth table, Boolean circuit, output for that circuit diagram, and Ladder logic diagram for mTOR, PTEN and AkT

RIP	IKK	NF-kB	Output
0	0	0	0
0	0	1	0
0	1	0	0
0	1	1	0
1	0	0	0
1	0	1	0
1	1	0	0
1	1	1	1

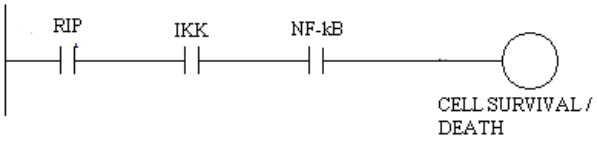
(*a*) Truth Table



(*b*) Boolean Circuit using Gates



(*c*) Output of Boolean circuit



(*d*) Ladder Logic Circuit

Fig 7. Showing truth table, Boolean circuit, output for that circuit diagram, and Ladder logic diagram for RIP, IKK and NF-KB.

4. Conclusion

We had successfully made computational model for cell survival/ death using three inputs such as TNF, EGF and insulin With that model we had made truth table, Boolean expression and logical circuit for each possible pathway. We then simulate the results of each path and then

combine all the results and get result of TNF, EGF and Insulin for its survival / death using universal gates and Ladder Logic.

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Abbreviations

AP-1, Activation Protein 1; ASK1, Apoptosis signal-regulating kinase 1; EGF, epidermal growth factor; EGFR, epidermal growth factor receptor; ERK, extracellular-regulated kinase; FADD, Fas-Associated protein with Death Domain; FKHR, Forkhead transcription factor; GLUT4, Glucose transport; Grb2, growth factor receptor-bound 2; GSK 3, Glycogen synthase kinase 3; HOG, High osmolarity glycerol; IGF, insulin-like growth factor; IκB, I Kappa B (nuclear factor of kappa light polypeptide gene enhancer in B-cells inhibitor); IKK, IκB kinase; IR, insulin receptor; IRS1, insulin receptor substrate 1; JNK1, c-jun NH_2 terminal kinase 1; MAP kinases, mitogen-activated protein kinases; MEK, mitogen-activated protein kinase and extracellular-regulated kinase kinase; MK2, mitogen-activated protein kinase-activated protein kinase 2; mTOR, mammalian target of rapamycin; NF-κB, nuclear factor-κB; PDK, Phi Delta Kappa; PI3K, phosphatidylinositol 3-kinase; p38, P38 mitogen-activated protein kinases**;** ptEGFR, phospho-to-total EGFR; ptAkT, phospho-to-total AkT; Rac, Ras-related C3 botulinum toxin substrate; SAPK/JNK , Stress-activated protein kinase/Jun-amino-terminal kinase; SH2, Src homolgy 2; SODD, Silencer of death domains; SOS, Son of Sevenless; SPICE, Simulation Program For Integrated Circuit Emphasis; TNF, tumor necrosis factor; TNFR1, tumor necrosis factor receptor 1; TNFR2, tumor necrosis factor receptor 2; TRADD, TNFR associated via death domain; TRAF2, TNF receptor associated factor 2, TSC, Tuberous sclerosis complex; XIAP, X-linked Inhibitor of Apoptosis Protein.