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SYSTEM MODELING OF CELL SURVIVAL AND CELL DEATH : A DETERMINISTIC MODEL USING FUZZY SYSTEM

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ABSTRACT

The extraction of some effective features that can represent the identities of different classes plays a critical factor for any classification problems involving the analysis of complex data. Fuzzy systems are an alternative to traditional notions of set membership and logic that has its origins in ancient Greek philosophy, and applications at the leading edge of Artificial Intelligence. Yet, despite its long-standing origins, it is a relatively new field, and as such leaves much room for development. In this paper we apply a fuzzy logic to the analysis of a large, systematic dataset describing the dynamics of cell signaling downstream of TNF, EGF and Insulin receptors regulating cell survival and cell death. Simulations based on fuzzy logic recapitulate most features of the data and generate several predictions involving pathway crosstalk and regulation. The system modeling for cell survival and cell death has been implemented using Fuzzy Logic Toolbox software with MATLAB which is technical computing software as a tool for solving problems. The results obtain will give information on how the input signals inducing cell survival/death should be modulated to achieve desire outputs and thus helps the experimentalists to design proposals regarding possible improvements to cell survival/ cell death.

KEYWORDS : TNF, EGF , Insulin, Fuzzy Logic, MATLAB

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1. INTRODUCTION

Computational modeling is useful as a means to assemble and test what we know about the proteins and networks. Models can help address key questions about the measurement, definition and function of proteomic networks. The ability of cells to sense their environment and decide to survive or die is dependent largely upon growth factors. This work examines signaling networks that control the survival/ death decision treated with combinations of three primary signals (Gaudet et al. 2000, Janes et al. 2005, Weixin et al. 2006) the pro death cytokine, *tumor necrosis factor-α* (TNF) (Brokhaus et al. 1990, Thoma et al. 1990) and the pro survival growth factors, *epidermal growth factor* (EGF) (Libermann et al. 1984, Normanno et al. 2006) and insulin (Lizcano et al. 2002, Morris 1997, 2003). Members of the TNF receptor super family play pivotal roles in numerous biological events in organisms. Ligand-mediated trimerization by corresponding homo- or heterotrimeric ligands, the TNF family ligands, causes recruitment of several intracellular adaptors, which activate multiple signal transduction pathways. While recruitment of death domain (DD) containing adaptors such as Fas associated death domain (FADD) and TNFR associated DD (TRADD) can lead to the activation of a signal transduction pathway that induces apoptosis, recruitment of TRAF family proteins can lead to the activation of transcription factors such as, NF-kappaB and JNK thereby promoting cell survival and differentiation as well as immune and inflammatory responses. Individual TNF receptors are expressed in different cell types and have a range of affinities for various intracellular adaptors, which provide tremendous signaling and biological specificities. In addition, numerous signaling modulators are involved in regulating activities of signal transduction pathways downstream of receptors in this superfamily. Epidermal growth factor (EGF) is a key growth factor regulating cell survival. Through its binding to cell surface receptors, EGF activates an extensive network of signal transduction pathways that include activation of the PI3K/AKT, RAS/ERK and JAK/STAT pathways. These pathways predominantly lead to activation or inhibition of transcription factors that regulate expression of both pro- and anti-apoptotic proteins effectively blocking the apoptotic pathway. In cancer, EGF signaling pathways are often dysfunctional and targeted therapies that block EGF signaling have been successful in treating cancers. In this review, we will discuss the EGF survival signaling network, how it cross-talks with the apoptotic signaling pathways and the therapeutic drugs targeting the EGF survival pathway used to treat cancers. When insulin is injected to a cell, various signal transductions occur as a reaction. There are various molecular transducers such as Insulin Receptor (IR), Insulin Receptor Substrate (IRS), Protein Kinase B (PKB, also known as Akt), and Extracellular Signal-Regulated Kinase (ERK), etc., in a cell. These transducers are phosphorylated under some stimulation and then deliver information to other transducers. In this study, we describe a downstream signaling pathway involving PI3K, JAK/STAT, MEK/ERK, mTOR and MAPK. These pathways predominantly lead to activation or inhibition of transcription factors that regulate expression of both pro- and anti-apoptotic proteins effectively blocking the apoptotic pathway.

In this work, we propose fuzzy logic (FL) as an approach to logic-based modeling with the easy interpretability of Boolean models but significant advantages including the ability to encode intermediate values for inputs and outputs. In 1965 Lotfi A. Zadeh published his seminal work "Fuzzy Sets" which described the mathematics of fuzzy set theory, (Riza et al. 1997, Driankov et al. 1993). This theory proposed making the membership function (or the values False and True) operate over the

range of real numbers (0.0, 1.0). New operations for the calculus of logic were proposed, and showed to be in principle at least a generalization of classic logic. We show that FL can encode probabilistic and dynamic transitions between network states so as to create simple and fairly realistic depictions of cell signaling networks. A key advantage of logic based approaches, also exemplified by FL, and is the ability to construct models ad hoc based on knowledge of network topology and data (Aldridge et al. 2009, Walsh et al. 2003).

2. FUZZY SYSTEM MODEL

A fuzzy expert system consists of four components namely, the fuzzifier, the interference engine, the defuzzifier, and a fuzzy rule base as shown in Figure 1.

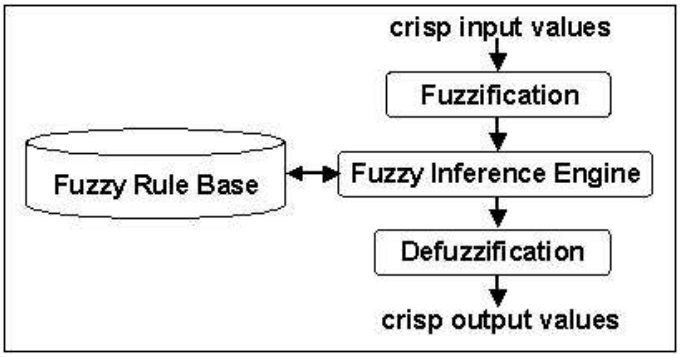

Figure 1
Fuzzy Expert System Model

In the fuzzifier, crisp inputs are fuzzified into linguistic values to be associated to the input linguistic variables. After fuzzification, the inference engine refers to the fuzzy rule base containing fuzzy IF-THEN rules to derive the linguistic values for the intermediate and output linguistic variables. Once the output linguistic values are available, the defuzzifier produces the final crisp values from the output linguistic values.

Fuzzy Inference System(FIS)

There are two types of Fuzzy inference system called a Mamdani type or Takagi-Sugeno-Kang. The main difference between Mamdani and Sugeno is that the Sugeno output membership functions are either linear or constant.

1. Fuzzify inputs:

Input is presented to the Fuzzifier (Riza et al. 1997, Driankov et al. 1993). The Fuzzifier then converts the numerical input – such as temperature, height, or any other measure, into a fuzzy value, that is, membership values in various groups and resolve all fuzzy statements in the antecedent to a degree of membership between 0 and 1. If there is only one part to the antecedent, this is the degree of support for the rule.

2. Application of fuzzy operator to multiple part antecedents: If there are multiple parts to the antecedent, fuzzy logic operators are apply and resolve the antecedent to a single number between 0 and 1 (Riza et al. 1997, Driankov et al. 1993). This is the degree of support for the rule. Two built-in AND methods are supported: *min* (minimum) and *prod* (product). Two built-in OR methods are also supported: *max* (maximum), and the probabilistic OR method *probor.* A single fuzzy if-then rule assumes the form *if* x *is* A *then* y *is* B where *A* and *B* are linguistic values defined by fuzzy sets on the ranges (universes of discourse) x and y, respectively. The if-part of the rule "*x* is *A*" is

called the *antecedent* or *premise*, while the then-part of the rule "y *is* B" is called the *consequent* or *conclusion*.

3. Apply implication method:
Use the degree of support for the entire rule to shape the output fuzzy set. The consequent of a fuzzy rule assigns an entire fuzzy set to the output. This fuzzy set is represented by a membership function that is chosen to indicate the qualities of the consequent. If the antecedent is only partially true, (i.e., is assigned a value less than 1), then the output fuzzy set is truncated according to the implication method. In general, one rule by itself doesn't do much good. What's needed are two or more rules that can play off one another. The output of each rule is a fuzzy set. The output fuzzy sets for each rule are then aggregated into a single output fuzzy set. Notice that as long as the aggregation method is commutative (which it always should be), then the order in which the rules are executed is unimportant. Three built-in methods are supported: *max* (maximum), *probor* (probabilistic OR), and *sum* (simply the sum of each rule's output set). Finally the resulting set is defuzzified*,* or resolved to a single number. There are five built-in methods supported: centroid, bisector, middle of maximum (the average of the maximum value of the output set), largest of maximum, and smallest of maximum.

3. FUZZY LOGIC IMPLEMENTATION

Among logic-based methods, the simplicity of Boolean models makes them attractive as a means to render biological networks. For example, a discrete-state representation of the level of phosphorylation of JNK might use three input edges TNF, TRAF2 and MAPK (where '1' means present or active, and '0' absent or inactive; Figure 2(*a*)). In Boolean logic, interactions among inputs are cast as combinations of elementary 'AND' gate that generate logic rules such as '(TNF AND TRAF2 AND MAPK)' and are most easily specified using gates (Figure 2(*b*)) and truth tables. Truth tables consist of lookup values for the outputs (consequent value) based on all possible combinations of input values (antecedents). Despite the appeal of Boolean models a two-state ''on-off'' representation of many biological signals is quite unrealistic. Working with FL models involves manipulating logic gates based on several adjustable parameters: (*i*) Membership functions (MFs) are used to assign values of inputs to a descriptive input class. (*ii*) MFs define the degree of membership (DOM) that quantifies the mapping between inputs and MFs and is always between 0 (no membership) and 1 (full membership). Fuzzy logic is so-named because inputs can have non-zero DOM to more than one MF, unlike discrete-state logic in which MFs and DOMs only take on values of 0 and 1. Figure 2(*c*) illustrates example MFs for Boolean and fuzzy logic models. (*iii*) The steepness of the membership functions is parameterized by the degree of fuzziness (note that Boolean logic models have a degree of fuzziness of 0). (*iv*) Logic rules relate the input state to the output state. In doing so, these rules encode how the input proteins regulate the activity of output protein. Once the logic rules are established, an FL gate is generated by first fuzzifying the inputs, a step that computes the DOM of each input state over the current input values and the pre-specified MFs. The degree of firing (DOF), then specifies whether a rule should be used (1) or not (0) as determined from the lowest DOM amongst the antecedents and the rule weight, a value between 0 and 1 that allows additional tuning of a rule's importance. In contrast to Boolean logic (BL) gates in which only one rule can fire for any set of input values (that is, only one row in the truth table is applied) (ldridge et al. (2009), FL gates allow multiple rules to fire to varying degrees (as defined by the DOF, Figure 2(*d*)). Defuzzification is the final step in which the superposition of multiple rules is resolved to determine the output value for the gate. Because of the flexibility of FL gates at

the input and output levels, intermediate levels of activity and complex processing functions can be modeled using networks similar in overall structure to familiar BL networks. However, flexibility also comes at the cost of additional free parameters; to minimize their numbers we use only a subset of available FL functions. This involves using few intermediate (between 0 and 1) rule weights or membership classes and allowing only one degree of fuzziness for all inputs in a given gate.

A model with three inputs TNF, EGF and Insulin was constructed by joining together or with some other protein shown in Figure.3. We have made the different pathways which define the cell survival and cell death. On the basis of the pathways we have made the truth table. Then we implemented those truth tables using Fuzzy logic.

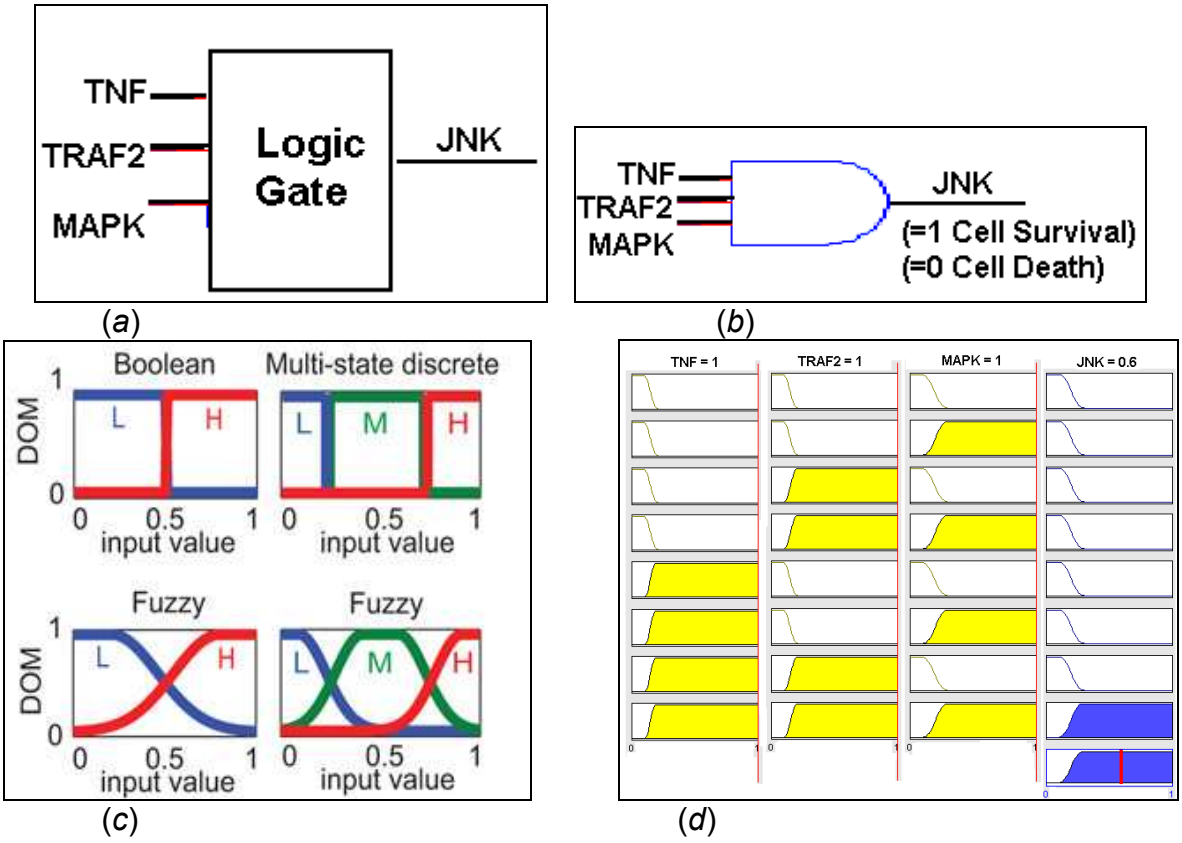

Figure 2

(*a*) Logic-based models use incoming edges to contain activity level of input or regulatory network species (for JNK, the inputs were TNF, TRAF2, and MAPK) with the logic gate at the node that performs the logic operation to update output signal (JNK) (*b*) A Boolean logic gate for JNK could be represented in terms of the logic statement "(TNF and TRAF2 and MAPK)", represented here in schematic form where the shape is an "AND-gate"). (*c*) To set up a FL gate, the first step is to assign membership functions (MFs) to the input variables. Each input variable has two or three membership functions ('L', 'M', and 'H' representing low, medium, and high states, respectively). An MF relates an input value to that state's degree of membership (DOM). MFs for Fuzzy and Boolean (2 MFs)/discrete multi-state (.2 MFs) logic forms are illustrated with the same state thresholds. (*d*) To set up a FL gate, the MFs for the inputs and for the outputs are defined. For simplicity, we use normalized

input and output values. Next, logic rules are listed as ‘‘if A (the antecedent), then B (the consequent)’’ using the input and output states as descriptors. Weights between 0 and 1 are assigned to each rule, which is helpful for rules that should have minor influence (e.g. rule 4).

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The rules for TNF, TRAF2, MAPK are each graphically listed with the outline of the membership functions specified for that rule’s antecedent. The consequent for each rule is also indicated by MF.

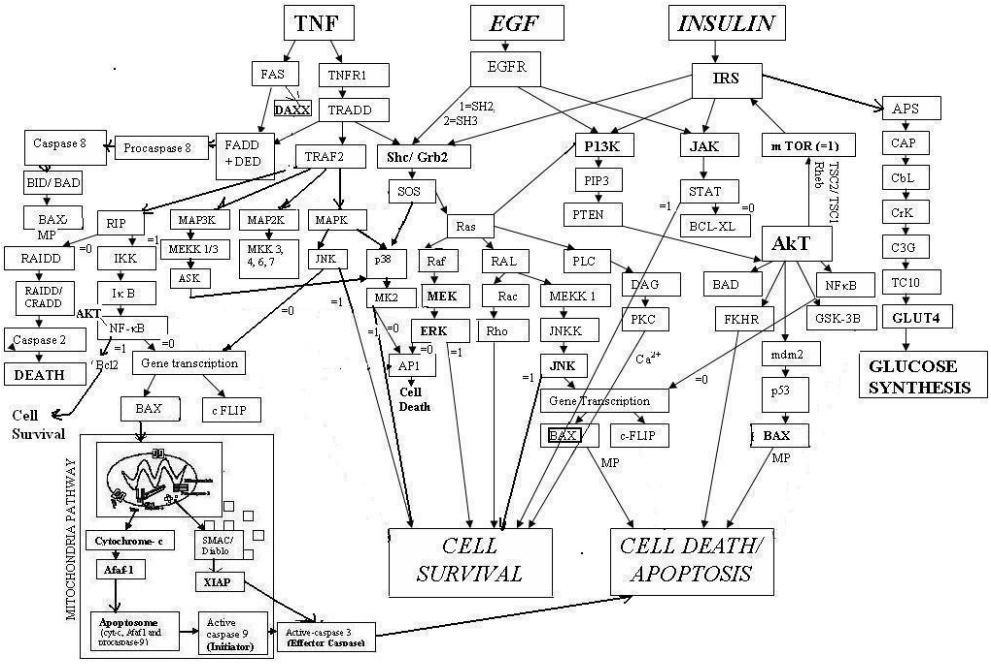


Figure 3
The original network diagram is adapted from Janes et al.(2005) and was used as a starting point to construct the FL gates

3.1 TUMOR NECROSIS FACTOR-α (TNF)*:* The TNF pathway is initiated by TNF/TNF-R1 complexes internalized into endocytic vesicles. At this intracellular level, the multi protein complexes associated to the receptors' tails modify and form the so-called Death Inducing Signaling Complex (DISC), whereby TRADD recruits FADD and pro-caspase-8, (Brokhaus et al. 1990, Thoma et al. 1990). This caspase then triggers the irreversible pathway leading to apoptosis. TRADD recruits TRAF2 and RIP. TRAF2 in turn recruits the multicomponent protein kinase IKK, enabling the serine-threonine kinase RIP to activate it. An inhibitory protein, IκBβ , that normally binds to NF-κB and inhibits its translocation, is phosphorylated by IKK and subsequently degraded, releasing NF-κB. NF-κB is a heterodimeric transcription factor that translocates to the nucleus and mediates the transcription of a vast array of proteins involved in cell survival and proliferation, inflammatory reponse, and anti-apoptotic factors. NF-κB is a family of transcription factors, which induce the expression of a wide variety of genes, especially those involved in survival, such as the Bcl-2 family member Bfl-1, and the caspase inhibitors c-IAP1 and c-IAP2.

The activation of MAPK kinase by tumor necrosis factor alpha (TNF-α) is found to be required for TNF- α induced apoptosis. This kinase is also involved in UV radiation induced apoptosis, which is thought to be related to cytochrome-c mediated cell death pathway. Of

the three major MAPK cascades, (Dudley et al. 1995, Zhou et al. 1995). TNF induces a strong activation of the stress-related JNK group, evokes moderate response of the p38- MAPK, and minimal activation of the classical ERKs. TRAF2 activates the JNK-inducing upstream kinases of MEKK1 and ASK1 (either directly or through GCKs and Trx, respectively), and these two kinases phosphorylate MKK7, which then activates JNK C-Jun N-terminal kinases (JNKs). The pathways involving TNF that regulates cell survival and cell death are as follows.

1) $TNF / TRAF2 / MAPK \rightarrow JNK\ (=1 \text{Cell Survival}, =0 \text{Cell Death});$ $(\text{Result Shown in Fig } 4(a))$

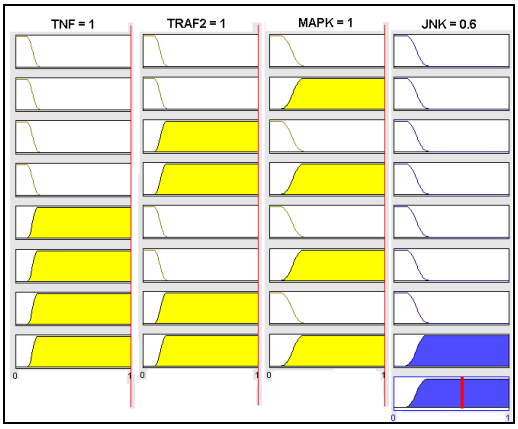
2) $TNF / MAPK / p38 \rightarrow MK2 (=1 \text{Cell Survival}, =0 \text{Cell Death});$ $(\text{Result Shown in Fig } 4(b))$

3) $TNF / RIP1 / IKK \rightarrow NF\kappa B (=1 \text{Cell Survival}, =0 \text{Cell Death});$ $(\text{Result Shown in Fig } 4(c))$

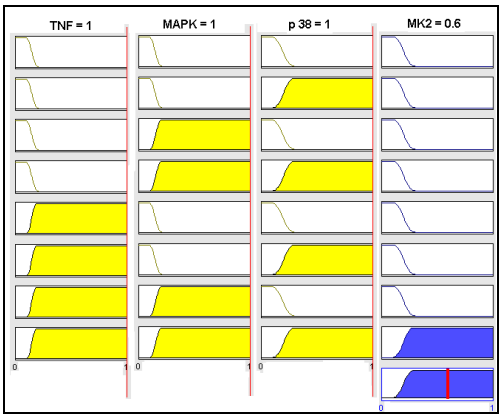
4) $TNF / RAS / MEK \rightarrow ERK (=1 \text{Cell Survival}, =0 \text{Cell Death});$ $(\text{Result Shown in Fig } 4(d))$

5) $TNF / FAS \rightarrow CASPASE 8\ (\text{Cell Death}).$ $(\text{Result Shown in Fig } 4(e))$

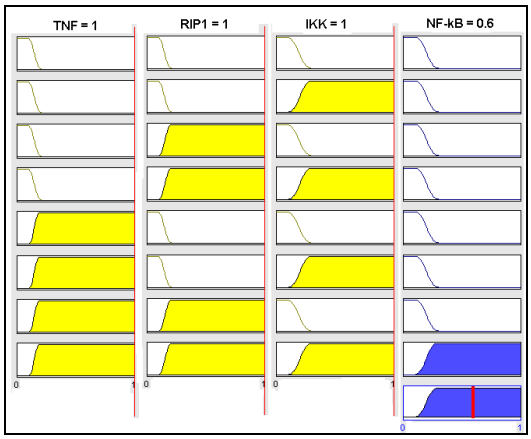
All these pathways have been using Fuzzy Tool box of MATLAB by taking data as: Type ='Mamdani', And Method ='Min', Or Method ='Max', Implication method ='Min', Aggregation Method ='Max', Defuzzification Method ='Centroid' shown in Fig 4 (*a*-*e*). Yellow filled boxes are treated as '1' i.e. high, while blank ones are '0' i.e. low. In last column, blue filled part in Fig 4(*a*, *b*, *c*, *d*) represents Cell Survival while Fig 4(*e*) represents Cell death.



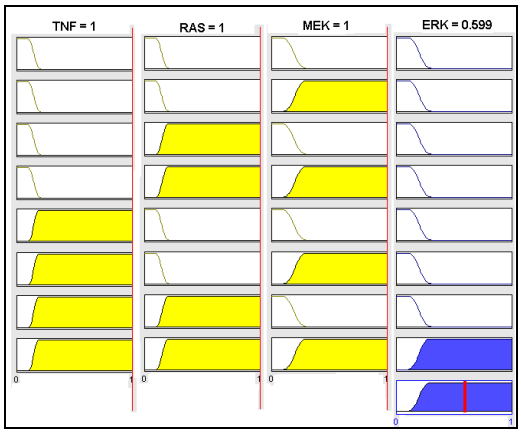
(*a*)



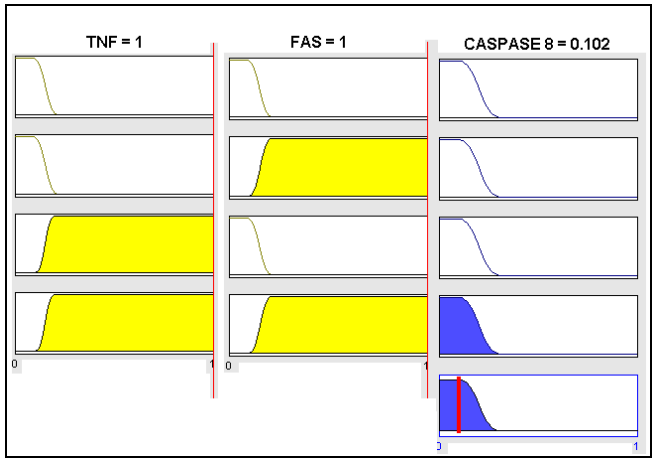
(*b*)



(*c*)



(*d*)



(*e*)

Figure 4

Showing FL output of each pathway of TNF. (a) Output for TNF/ TRAF2/ MAPK = JNK pathway. (b) Output for TNF/ MAPK/ p38 = MK2 pathway. (c) Output for TNF/ RIP1/ IKK = NF-κB pathway. (d) Output for TNF/ RAS/ MEK = ERK pathway. (e) Output for TNF/ FAS = Caspase 8 pathway.

3.2 EPIDERMAL GROWTH FACTOR *:* EGF transduces the cellular signal upon binding to cell receptors (Jelinek et al. 1994) Activation of the cell receptor takes place by transphosphorylation at tyrosine residue. 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. The EGF signal is terminated primarily through endocytosis of the receptor-ligand complex. The contents of the endosomes are then either degraded or recycled to the cell surface. 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 (Hallberg et al 1994, Eyers et al. 1998). RAF-1 subsequently phosphorylates MEK1 and MEK2 which activate respectively ERK1 and ERK2. This pathway results in cell proliferation and in the increased transcription of Bcl2 family members and inhibitors of apoptosis proteins (IAPs), thereby promoting cell survival. Mitogenic sigalling increases the rate of translation of selective mRNAs. 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 (Dudley et al. (1995), Zhou et al. (1995)). The first is mediated by mitogenic and differentiation signals. The other two respond to stress and inflammatory cytokines. The ERK pathway responds to mitogen activation. In the JNK/SAPK pathway SAPK stands for stress activation protein kinase and within this class the JNK (Jun N-terminal kinases) is a subfamily. In the p38/HOG pathway HOG stands for high osmolarity glycerol where as the p38 proteins are a subfamily. Each of these pathways led to the dual phosphorylation of MAP kinase family members responsible for activation of transcription factors. Cytokines and growth factors activate the mitogen-activated protein (MAP) kinase pathways resulting in the stimulation of ERK1/2, c-Jun N-

terminal kinases and p38 kinases which in turn activate transcription factors like AP-1 and ATF-2.

EGF also promotes cell survival through the activation of PI3 kinase/ AkT signaling (Gaudet et al. (2000), Janes et al. (2005), Weixin et al. (2006)). 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. The catalytic subunit of PI3-kinase in turn phosphorylates phosphatidylinositol (4, 5) biphosphate (PtdIns (4, 5)P_2) leading to the formation of PtdIns(3,4,5)P_3. 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 (Dohoon et al. (2002)). Intermediate transcription factors involved in this process are NFκB and CREB. AKT also activates mammalian target of rapamycin (mTOR) (Laura et al. (2004), which promotes protein synthesis through p70 ribosomal S6 kinase (p70s6k) and inhibition of eIF-4E binding protein (4E-BP1). Collectively, these processes all promote cell growth and survival in response to EGF.

Another signaling cascade initiated by EGF is the JAK/STAT pathway, which is also implicated in cell survival responses (Kisseleva et al. (2002)). 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. Following are the pathways of EGF regulating cell survival/ cell death.

$$1)\ EGF/RAS/MEK \rightarrow ERK(=1\,\text{Cell Survival}, =0\,\text{Cell Death}); \quad (\text{Result Shown in Fig } 5(a))$$

$$2)\ EGF/RAS/MEKK1 \rightarrow JNK(=1\,\text{Cell Survival}, =0\,\text{Cell Death}); \quad (\text{Result Shown in Fig } 5(b))$$

$$3)\ EGF/PI3K/AKT \rightarrow NF\kappa B(=1\,\text{Cell Survival}, =0\,\text{Cell Death}); \quad (\text{Result Shown in Fig } 5(c))$$

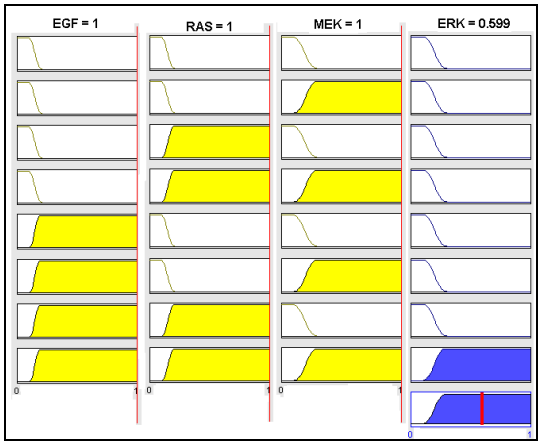
$$4)\ EGF/PI3K/AKT \rightarrow BAD(=1\,\text{Cell Survival}, =0\,\text{Cell Death}); \quad (\text{Result Shown in Fig } 5(d))$$

$$5)\ EGF/PI3K/AKT \rightarrow FKHR(\text{Cell Death}); \quad (\text{Result Shown in Fig } 5(e))$$

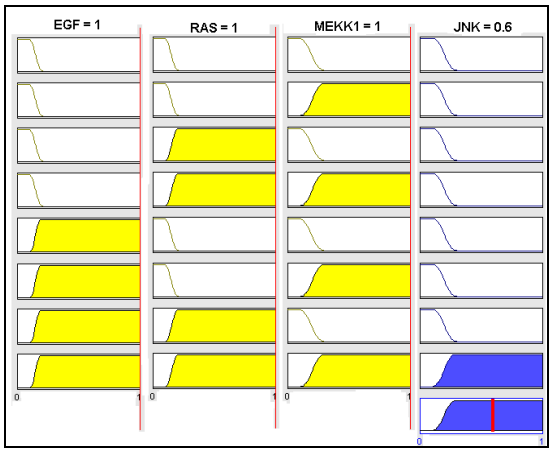
$$6)\ EGFR/JAK \rightarrow STAT(=1\,\text{Cell Survival}, =0\,\text{Cell Death}); \quad (\text{Result Shown in Fig } 5(f))$$

$$7)\ \text{EGFR}/\ p38 \rightarrow MK2(=1\,\text{Cell Survival}, =0\,\text{Cell Death}). \quad (\text{Result Shown in Fig } 5(g))$$

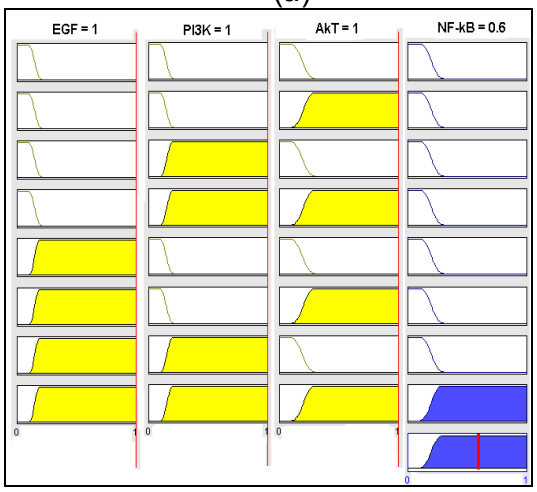
We have implemented different pathways using Fuzzy Tool box of MATLAB by taking data as: Type ='Mamdani', And Method ='Min', Or Method ='Max', Implication method ='Min', Aggregation Method ='Max', Defuzzification Method ='Centroid' shown in Fig 5 (*a*-*g*). Yellow filled boxes are treated as '1' i.e. high, while blank ones are '0' i.e. low. In last column, blue filled part in Fig 5(*a*, *b*, *c*, *d*, *f*, *g*) represents Cell Survival while Fig 5 (*e*) represents cell death.


EGF = 1
RAS = 1
MEK = 1
ERK = 0.599

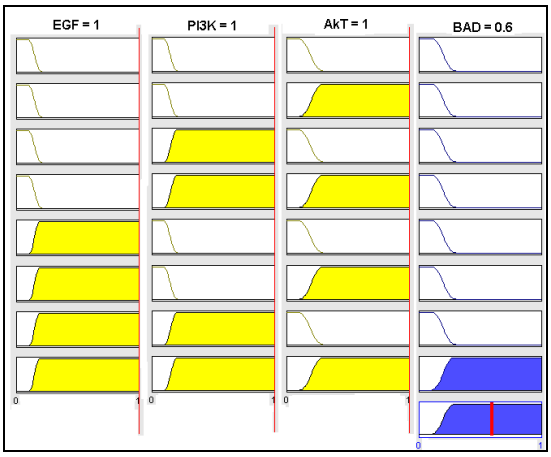
(*a*)


EGF = 1
RAS = 1
MEKK1 = 1
JNK = 0.6

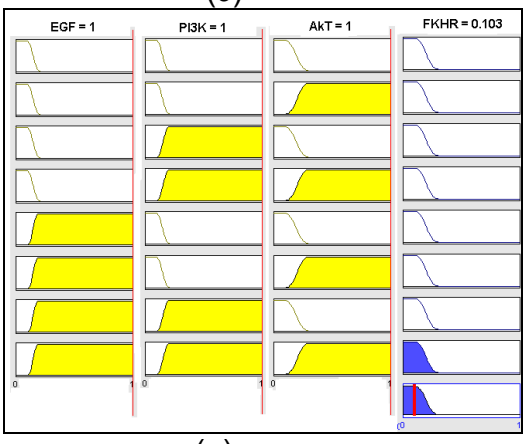
(*b*)


EGF = 1
PI3K = 1
AkT = 1
NF-kB = 0.6

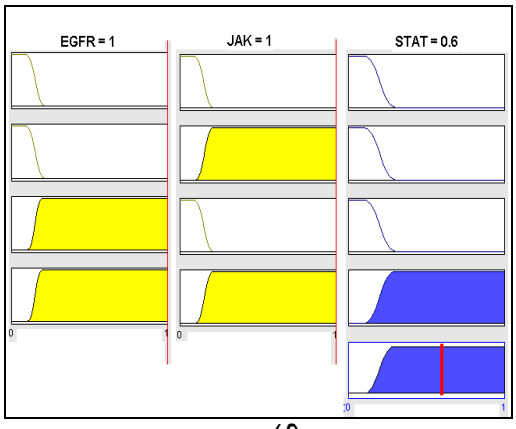
(*c*)


EGF = 1
PI3K = 1
AkT = 1
BAD = 0.6

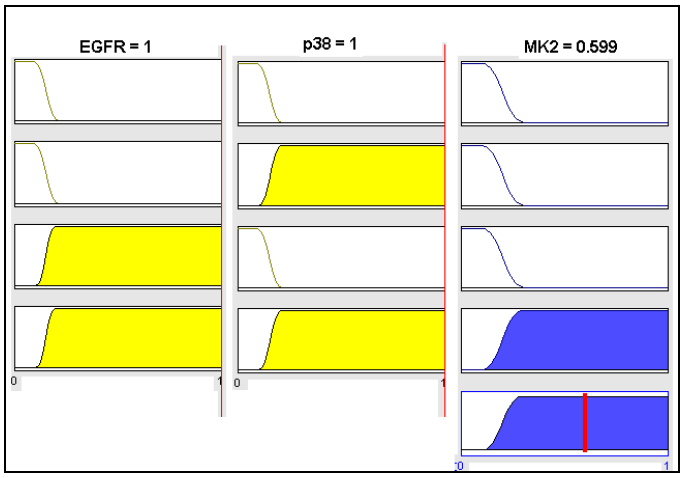
(*d*)


EGF = 1
PI3K = 1
AkT = 1
FKHR = 0.103

(*e*)


EGFR = 1
JAK = 1
STAT = 0.6

(*f*)



(*g*)

Figure 5

Showing FL output of each pathway of EGF. (a) Output for EGF/ RAS/ MEK = ERK pathway; (b) Output for EGF/ RAS/ MEKK1= JNK pathway; (c) Output for EGF/PI3K/ AkT = NF-κB pathway; (d) Output for EGF/PI3K/ AkT = BAD pathway; (e) Output for EGF/PI3K/ AkT = FKHR pathway; (f) EGFR/ JAK = STAT Pathway; (g) EGFR/ p38 = MK2 Pathway.

3.3 INSULIN : Insulin is a hormone (Ullrich et al. (1990)) that regulates the amount of glucose (sugar) in the blood and is required for the body to function normally. This protein binds to its receptor (Zierath et al. (2000), Kahn (1988)) the insulin receptor, on cell membrane, which initiates a process of signal transduction. The insulin receptor (Withers (2001), Basera (1995), Blakesley et al. (1996)) is an insulin-dependent tyrosine kinase. Insulin binds to the extracellular α-subunit of the receptor and induces a conformational change that brings the α-subunits closer together. This leads to a rapid autophosphorylation of the receptor. This, then allows other intracellular proteins to bind to the intracellular domain of the receptor, and become phosphorylated (Yenush et al. (1996)). PI3K is activated by insulin, insulin-like growth factor-1 and other growth factors. PI3K is a heterodimeric lipid kinase with a broad range of cellular functions, including growth and differentiation, synthesis and degradation of carbohydrates, proteins and lipids, and membrane trafficking. PI3K consists of a regulatory subunit that associates with a catalytic subunit. The regulatory subunit binds the IRSs, whereas the catalytic subunit phosphorylates in the membrane. PDK (phosphoinositide-dependent protein kinase)/Akt Akt (protein kinase B, c-Akt) are one of the serine/threonine kinases downstream of PI3K (Gaudet et al. (2000), Janes et al. (2005), Weixin et al. (2006)). Intermediate transcription factors involved in this process are NFκB and JNK. MAP kinases are actually a family of protein kinases that are widely distributed and are found in all eukaryotic organisms. These can be classified into three main functional groups. The first is mediated by mitogenic and differentiation signals. The other two respond to stress and inflammatory cytokines. The ERK pathway responds to mitogen activation. In the p38/HOG pathway HOG stands for high osmolarity glycerol where the p38 proteins are a subfamily. Each of these pathways led to the dual phosphorylation of MAP kinase family members responsible for activation of transcription factors. Cytokines and growth factors activate the mitogen-activated protein (MAP) kinase pathways resulting in the stimulation of ERK1/2, c-Jun N-terminal kinases and p38 kinases which in turn activate transcription factors like AP-1 and ATF-2.

Using cell biological, biochemical, genomic, and proteomic approaches, we are uncovering the complex molecular understanding of a signaling network centered around a G protein switch involving 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 (Asnaghi et al. (2004), Ogawa et al. (1998)) 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. Although genetic analysis has demonstrated that members of the winged helix, or forkhead, family of transcription factors play pivotal roles in the regulation of cellular differentiation and proliferation (Barthel et al. (2001), Schmoll et al. (2000)), both during development and in the adult, little is known of the mechanisms underlying their regulation. Another signaling cascade initiated by Insulin is the JAK/STAT pathway, which is also implicated in cell survival responses. 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. Pathways which lead to cell survival/death using INSULIN are as follows:

$$1)\ Insulin / RAS / MEK \rightarrow ERK\,(=1\,\text{Cell Survival}, =0\,\text{Cell Death}); \quad (\text{Result Shown in Fig } 6(a))$$

$$2)\ Insulin / RAS / MEKK1 \rightarrow JNK\,(=1\,\text{Cell Survival}, =0\,\text{Cell Death}); \quad (\text{Result Shown in Fig } 6(b))$$

$$3)\ Insulin / PI3K / AKT \rightarrow NF\kappa B\,(=1\,\text{Cell Survival}, =0\,\text{Cell Death}); \quad (\text{Result Shown in Fig } 6(c))$$

$$4)\ Insulin / PI3K / AKT \rightarrow BAD\,(=1\,\text{Cell Survival}, =0\,\text{Cell Death}); \quad (\text{Result Shown in Fig } 6(d))$$

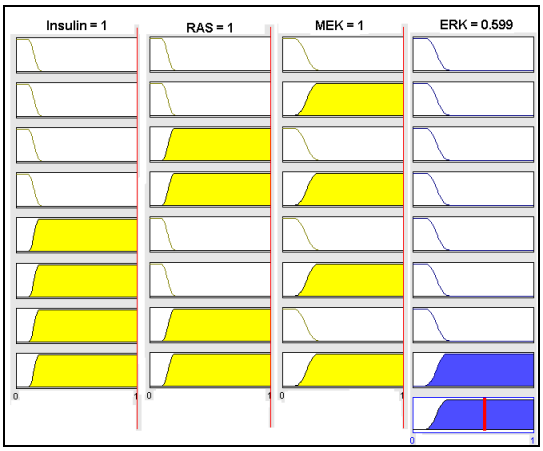
$$5)\ Insulin / PI3K / AKT \rightarrow FKHR\,(\text{Cell Death}); \quad (\text{Result Shown in Fig } 6(e))$$

$$6)\ IRS / JAK \rightarrow STAT\,(=1\,\text{Cell Survival}, =0\,\text{Cell Death}); \quad (\text{Result Shown in Fig } 6(f))$$

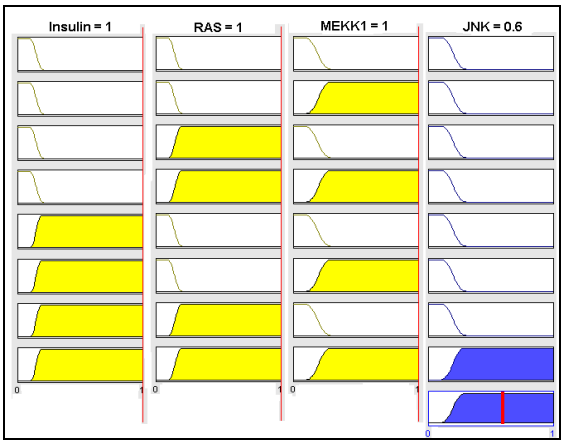
$$7)\ IRS / p38 \rightarrow MK2\,(=1\,\text{Cell Survival}, =0\,\text{Cell Death}); \quad (\text{Result Shown in Fig } 6(g))$$

$$8)\ Insulin / AKT / mTOR \rightarrow IRS\,(=1\,\text{Cell Survival}, =0\,\text{Cell Death}). \quad (\text{Result Shown in Fig } 6(h))$$

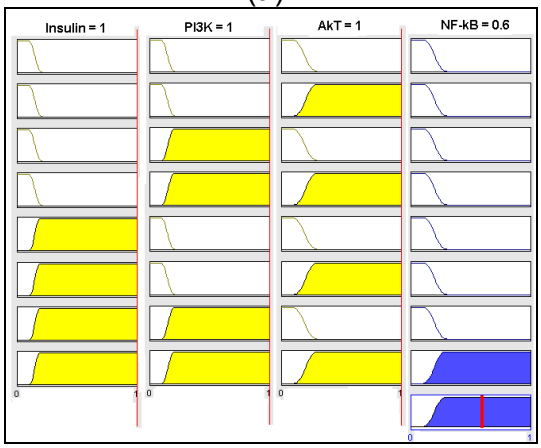
We have implemented different pathways using Fuzzy Tool box of MATLAB by taking data as: Type ='Mamdani', And Method ='Min', Or Method ='Max', Implication method ='Min', Aggregation Method ='Max', Defuzzification Method ='Centroid' shown in Fig 6 (*a*-*h*). Yellow filled boxes are treated as '1' i.e. high, while blank ones are '0' i.e. low. In last column, blue filled part in Fig 6(*a*, *b*, *c*, *d*, *f*, *g*, *h*) represents Cell Survival while Fig 6 (*e*) represents Cell death.


Insulin = 1
RAS = 1
MEK = 1
ERK = 0.599

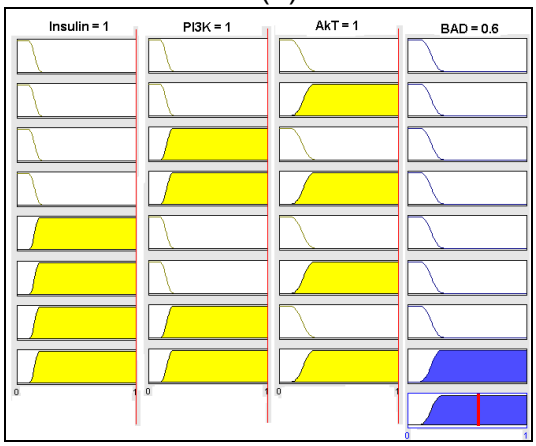
(*a*)


Insulin = 1
RAS = 1
MEKK1 = 1
JNK = 0.6

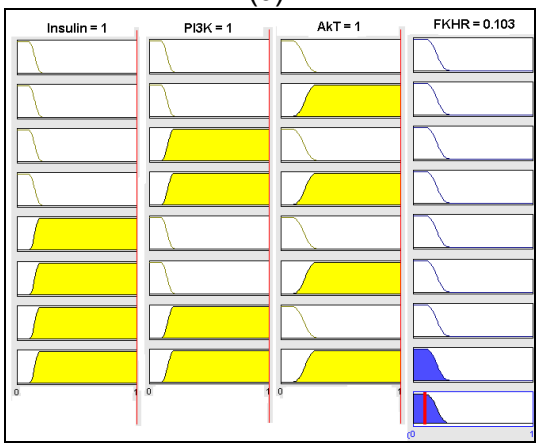
(*b*)


Insulin = 1
PI3K = 1
AkT = 1
NF-kB = 0.6

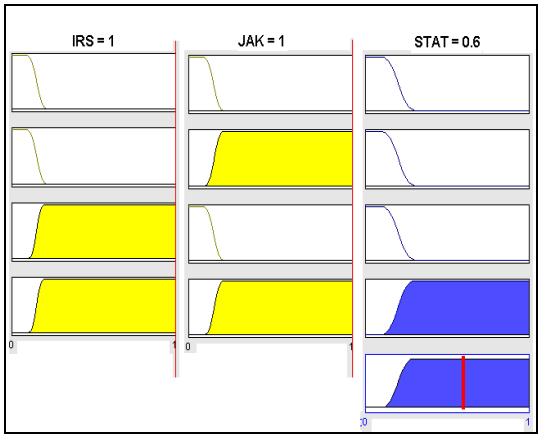
(*c*)


Insulin = 1
PI3K = 1
AkT = 1
BAD = 0.6

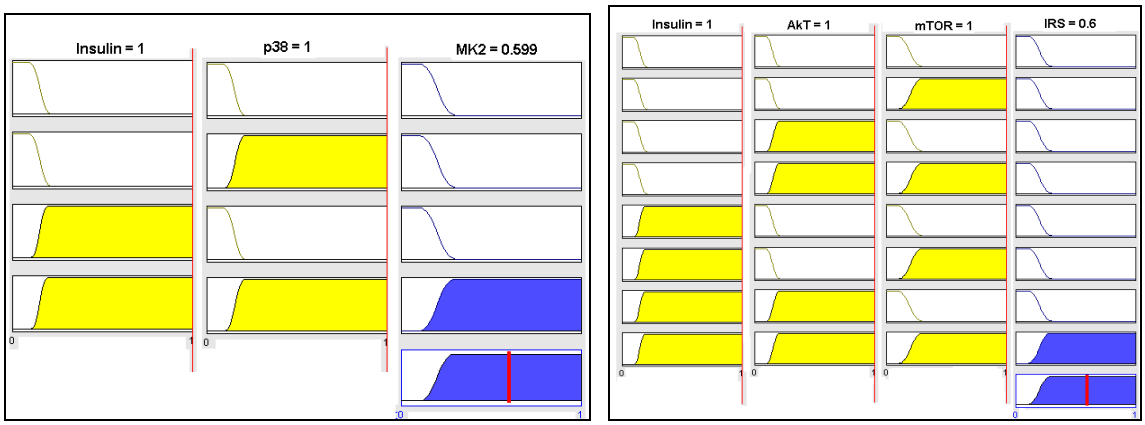
(*d*)


Insulin = 1
PI3K = 1
AkT = 1
FKHR = 0.103

(*e*)


IRS = 1
JAK = 1
STAT = 0.6

(*f*)



(*g*) (*h*)

Figure 6

Showing FL output of each pathway of Insulin. (a) Output for Insulin/ RAS/ MEK = ERK pathway; (b) Output for Insulin / RAS/ MEKK1= JNK pathway; (c) Output for Insulin/PI3K/ AkT = NF-κB pathway; (d) Output for Insulin/PI3K/ AkT = BAD pathway; (e) Output for Insulin/PI3K/ AkT = FKHR pathway; (f) IRS/ JAK = STAT Pathway; (g) Insulin/ p38 = MK2 Pathway; (h) Insulin/ AkT /mTOR = IRS Pathway

4. CONCLUSION

We have demonstrated that the Fuzzy logic can be applied to predict the cell survival/ death with a high level of accuracy using input TNF, EGF and Insulin. The signaling pathway has reproduced experimental data with accurate. Understanding the nature of signaling networks that control the cell survival/ death is very significant and theoretical calculations, in particular the simulation process developed using FUZZY, seen to be a proper tool for gaining such understanding. The results obtain will give information on how the input signals inducing cell survival/ death should be modulated to achieve desire outputs and thus helps the experimentalists to design proposals regarding possible improvements to cell survival/ cell death.

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