Signaling Pathways and Model Checking in the Pancreatic Cancer Studies

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Outline

- Introduction
- Signaling Pathways
- HMGB1 and Pancreatic Cancer
- Model Checking
- Future Work
- Acknowledgement
Oncoprotein & Tumor-suppressor Protein

- **Oncoproteins** stimulate cell growth under normal conditions.

- Cells with **mutant oncoproteins** continue to grow (refuse to die) even when they are receiving no-growth signals.

- Some examples of oncoproteins are **RAS, AKT, MDM2**.

- **Tumor-suppressor proteins** can inhibit the cell cycle progress or promote **apoptosis** *(programmed cell death)*.

- In normal cell, oncoproteins are regulated by the tumor-suppressor proteins.

- Some examples of tumor-suppressor proteins are **P53, RB, PTEN, INK4A, and ARF**.
The Hallmarks of Cancer

R.A. Weinberg, The Hallmarks of Cancer
Genetic Mutations in Pancreatic Cancer

• KRAS mutations exist in about 90% of pancreatic cancer

• INK4A & ARF mutations exist in over 80% of pancreatic cancer

• P53 is mutated in over 50% of pancreatic cancer

• PTEN loss exists in over 40% of pancreatic cancer

• AKT overexpressed in over 20% of pancreatic cancer
I. The P53-MDM2 Pathway

• Important Proteins:
  1. P53
  2. MDM2
  3. PI3K
  4. PTEN
  5. AKT

• Positive and negative feedback loops in the P53-MDM2 pathway
The P53 Protein

• **P53** is a tumor suppressor and regulates the cell cycle by integrating numerous signals that control cell life and death.

• P53 is mutated in more than **50% of pancreatic cancers**.

• P53 is a transcription factor for many genes including the pro-apoptosis and anti-apoptosis genes, e.g., Bax, mdm2.

• P53 is short-lived and expressed at very low levels in NORMAL cells. **BUT**, P53 becomes stable and accumulates if the cell has DNA damage.

• Functions of P53:
  - **Induces cell cycle arrest**: P21, etc.
  - **DNA repair**: P53R2
  - **Initiates apoptosis – Programmed Cell Death**: Bax, etc.
The MDM2 Protein

- MDM2 is an oncoprotein, it is a P53 negative regulator.
- MDM2 is one of P53’s transcription targets.
- Unphosphorylated MDM2 can only reside in the cytoplasm.
- Phosphorylated MDM2 could enter the nucleus and bind to P53 to inhibit P53’s transcription activity, and target P53 for degradation.
- When DNA is damaged, MDM2-P53 interaction decreases, P53 is activated.

L. Mayo, D. Donner, Trends in Biochemical Sciences, 2002
The Proteins PI3K and PTEN

- PI3K is an oncoprotein, activated by some growth factors (GF).
- PI3K can phosphorylate the lipid PIP2 to PIP3, then activate the AKT signaling pathway.
- PTEN is a tumor suppressor protein. It is also one of P53’s transcription targets.
- PTEN can dephosphorylate PIP3 back to PIP2, then, inhibit the AKT signaling pathway.
- PTEN loss occurs in more than 40% of pancreatic cancers.
The Protein AKT

- **Protein AKT**, also called protein kinase B (PKB), is an *oncoprotein*. It is overexpressed in many types of cancer.

- **AKT overexpression** exists in more than 20% of pancreatic cancers.

- AKT is phosphorylated and activated by PIP3.

- AKT controls cell survival by regulating many downstream proteins or signaling pathways, e.g. NFκB, MDM2.

- AKT can phosphorylate and activate MDM2 to block apoptosis
P53-MDM2 Pathway

**Negative feedback loop**

P53 → MDM2 → P53

**Positive feedback loop**

P53 → PTEN → PIP3 → AKT → MDM2 → P53

GF → PI3K → PIP3 → AKT → MDM2

PTEN

Damaged DNA → Apoptosis

P53
II. RB-E2F Pathway

- Cell Cycle Introduction
- Important Proteins:
  1. CYCLIN
  2. CDK
  3. RB
  4. E2F
  5. INK4A
  6. ARF

- RB-E2F Pathway
Phases of the Cell Cycle

- **G0**: resting, non-proliferating state.
- **G1**: cell is active and continuously growing, but **no DNA replication**.
- **S (synthesis)**: DNA replication.
- **G2**: continue cell growth and synthesize proteins.
- **M (mitosis)**: cell divides into two cells.

*Weinberg, The Biology of Cancer*
The Proteins Cyclin and CDK

- **Cyclins** control the progression of the cell cycle by activating the cyclin-dependent kinases (CDKs).

- Both cyclins and CDKs are **oncoproteins**.

- In early G1 phase, cyclin D assembles with CDK4/6 to form cyclin D-CDK4/6 complex.

- Cyclin D-CDK4/6 complex is **overexpressed** in most cancers.

- **Disruption** of cyclin D-CDK4/6 complexes could prevent tumor progression.

- **Cyclin E-CDK2** complex plays an important role in G1-S phase transition.
The Proteins RB and E2F

- **RB** is a *tumor suppressor*:
  - disrupts the transition from phases G1 to S in the cell cycle
  - prevents the replication of damaged DNA
  - absent, or present in a *defective* form, in many tumor cells.

- **E2F** is an *oncoprotein*, it *promotes the transition* from phase G1 to S in the cell cycle, and it is *modulated* by RB proteins.

- In G1 phase, *unphosphorylated* RB protein binds to E2F to inhibit its transcription activity.

- E2F is activated after RB is *phosphorylated* by some oncoproteins (cyclin D-CDK4/6) which is activated in RAS-ERK pathway.
The Protein INK4A and ARF

- **INK4A** is a tumor suppressor.
  - inhibits the activity of cyclin D-CDK4/6 complex
  - prevents phosphorylation of RB and inactivates E2F

- INK4A loss occurs in over 80% of pancreatic cancers.

- **ARF** is also a tumor suppressor. It is activated by some overexpressed oncoproteins, eg. E2F.

- ARF inhibits the activity of MDM2, thus preventing P53 degradation.

- ARF mutation occurs in over 80% of pancreatic cancers.
**RB-E2F Pathway**

- RAS-ERK pathway activates Cyclin D transcription.
- Cyclin D-CDK4 phosphorylates RB, leading to the activation of E2F protein.
- Oncoprotein Myc activates E2F.
- E2F activates the transcription of Cyclin E, facilitates the G1-S transition. Cyclin E can further inhibit RB.
- ARF is activated by over-expressed oncprotein E2F.
- ARF can promote MDM2 degradation.
HMGB1 and Pancreatic Cancer

- HMGB1 Protein
- HMGB1 and Pancreatic Cancer
The Protein HMGB1

- High-Mobility Group Protein 1 (HMGB1):
  - DNA-binding protein and regulates gene transcription
  - released from damaged or stressed cells, etc.
- HMGB1 activates RAGE or TLR2/4
  - RAGE: Receptor for Advanced Glycation End products.
  - TLR: Toll-like receptor

- RAGE/TLR activation can activate NFκB and RAS signaling pathways which causes inflammation or tumorigenesis.
HMGB1 and Pancreatic Cancer
(Lotze et al., UPMC)

Apoptosis: “programmed” cell death

Experiments with pancreatic cancer cells:
- Overexpression of HMGB1/RAGE is associated with diminished apoptosis, and greater cancer cell survival.
- Knockout of HMGB1/RAGE leads to increased apoptosis, and decreased cancer cell survival.
Model Checking

• Models
  1. Ordinary Differential Equation Model
  2. BioNetGen Model
  3. Boolean Network Model

• Model Checking
ODE Model for P53-MDM2 Pathway

\[
\frac{d}{dt} \text{PIP}_3(t) = k_3 \text{PI3K}_a(t) \text{PI2}(t) - d_3 \text{PTEN}(t) \text{PI3}(t)
\]

\[
\frac{d}{dt} \text{AKT}_p(t) = k_4 \text{PI3}(t) \text{AKT}(t) - d_4 \text{AKT}_p(t)
\]

\[
\frac{d}{dt} \text{PTEN}(t) = s_p + \frac{k_5 \text{P53}(t)^3}{K^3 + \text{P53}(t)^3} - d_5 \text{PTEN}(t)
\]

Hill function

\[
\frac{d}{dt} \text{MDM2}(t) = s_m + \frac{k_6 \text{P53}(t)^3}{K_m + \text{P53}(t)^3} + d_{\tau_p} \text{MDM2}_p(t)
\]

\[
-k_7 \text{AKT}_p(t) \text{MDM2}(t) - d_6 \text{MDM2}(t)
\]
# PI3K phosphorylates PIP2
- PI3K + PIP2 → PI3K + PIP3    \( p_1 \)

# PTEN dephosphorylates PIP3
- PTEN + PIP3 → PTEN + PIP2    \( d_1 \)

# P53-dependent production of PTEN
- P53(c~p) → P53(c~p) + PTEN Hill(d2,K,3)

# PIP3 phosphorylates AKT
- PIP3 + AKT(a~U) → PIP3 + AKT(a~p)

- AKT(a~p) + MDM2(b~U) → AKT(a~p) + MDM2(b~p)    \( p_2 \)

# MDM2p drives P53 degradation
- MDM2(b~p) + P53(c~U) → MDM2(b~p)    \( d_5 \)

# P53 synthesis
- I() → I() + P53(c~U)    \( s_0 \)
Stochastic Simulation I
ARF loss and overexpression of PI3K

ARF inhibits MDM2 and P53 accumulates
Statistical Model Checking

Set BayesFactor threshold $T = 10,000$, based on Bayesian hypothesis testing, it stops when probability of error $<0.0001$

1. *P53 concentration increase when DNA is damaged.*

- **Property 1**: $\Pr_{\geq0.9} \left[ F_{10,000}^{10,000} (P53 > 180,000) \right]$
  Result 1: Accepted to be True: 122 Satisfiable, 5 Unsatisfiable

2. *P53 protein concentration remains in a low level in the normal cell.*

- **Property 2**: $\Pr_{\geq0.9} \left[ F_{60,000}^{60,000}(G_{20,000}^{20,000} (P53 < 50,000)) \right]$
  Result 2: Accepted to be True: 43 Satisfiable, 0 Unsatisfiable
Some update rules:

\[ \text{PI3k}(t+1) = \text{RAC1}(t) \mid \text{RAS}(t) \]

\[ \text{IKK}(t+1) = (\text{TAB1}(t) \mid \text{AKT}(t) \mid \text{ERK}(t)) \land \neg \text{A20}(t) \]

\[ \text{E2F}(t+1) = \text{Myc}(t) \land \neg \text{RB}(t) \]

\[ \text{RB}(t+1) = \neg \text{CyclinD}(t) \land \neg \text{CyclinE}(t) \]

\[ \text{CyclinD}(t+1) = (\text{AP1}(t) \mid \text{Myc}(t)) \land \neg \text{INK4a} \land \neg \text{P21} \]

\[ \text{PIP3}(t+1) = \text{PI3K}(t) \land \neg \text{PTEN}(t) \]

\[ \text{P53}(t+1) = \neg \text{MDM2}(t) \]

\[ \text{MDM2}(t+1) = \text{AKT}(t) \land \neg \text{ARF}(t) \]
Assume $INK4A = 0$ ($INK4A$ loss in pancreatic cancer)

- HMGB1 will activate the cell proliferation in the future:
  $AF(\text{Proliferate})$: True
- If RAS is overexpressed, CyclinE will be activated in the future
  $A(\text{RAS} \to A(\text{CyclinE}))$: True
- HMGB1 can activate E2F while passing by AKT
  $E(\text{AKT} \& EF(\text{E2F}))$: True
- ERK is not activated before E2F is activated:
  $E(\sim\text{ERK} \cup \text{E2F})$: False
- HMGB1 can inhibit Apoptosis in the future
  $EF(\sim\text{Apoptosis})$: True
Inference from Model Checking

Assume \( INK4A = 1 \) (NO \( INK4A \) mutation)

1. \( CyclinD = (Myc | AP1) | \neg INK4A \)
   - HMGB1 will activate E2F in the future:
     \[ AF(E2F): \text{True} \]
     HMGB1 and its effectors have a stronger effect than \( INK4A \)

2. \( CyclinD = (Myc | AP1) \& \neg INK4A \)
   - \( AF(E2F): \text{False} \)
     \( INK4A \) has a stronger effect than HMGB1 and its effectors:
     HMGB1 can not activate E2F.

Model checking can help to rule out or modify some models which do not satisfy the properties abstracted from experiment.
Future Work

• Asynchronous Boolean Network Model for HMGB1: protein mutations occur at different stages of pancreatic cancer

• Apply SMV to larger cell cycle boolean network: e.g., Kohn map, etc.

• Probabilistic Boolean Network Model and Statistical Model Checking

• In collaboration with experimental cancer biologist, estimate important parameters for the Non-boolean models.
Kohn Map

Figure 6B: The p53-Mdm2 and DNA repair regulatory network (version 2p - May 19, 1999)

Kurt W. Kohn, Molecular Biology of the Cell 1999
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Appendix
PanINs (Pancreatic intraepithelial neoplasias), represent progressive stages of neoplastic growth
III. RAS Pathways

• Important Proteins
  1. RAS
  2. RAF
  3. MEK
  4. MAPK (ERK1/2)

• RAS Pathways
The Protein RAS

- Protein **RAS** relays signals from outside the cell to the nucleus. Activation of RAS signaling causes **cell growth and survival**.

- RAS family has **three members**: HRAS, KRAS, NRAS.

- **KRAS mutations** increase with disease progression, and are found in more than 90% of pancreatic cancers.

- RAS is **activated** when it binds to **GTP** (Guanosine Triphosphate) which is catalyzed by **GEFs** (Guanine nucleotide Exchange Factors), **inactivated** if bound to **GDP** (Guanosine Diphosphate).

- **Aberrant signaling** through RAS pathways occurs if RAS is mutated or some growth-factor-receptor tyrosine kinases (EGFR, etc.) are over-expressed, or mutations of RAS effectors (RAF, MEK, PI3K).
The Proteins RAF, MEK and MAPK

- RAF protein usually exists in the cytoplasm. When activated by GTP-RAS protein it relocates to the plasma membrane.

- The activated RAF can phosphorylate and activate the MEK proteins (mitogen-activated protein kinase kinases (MAPKK)).

- The activated MEKs can phosphorylate and activate protein MAPKs (ERK1/2)

- The activated ERK can phosphorylate transcription factors such as ELK1, AP-1 and ETS, which activate the expression of the regulatory proteins, including Cyclin D protein, that enable progression of the cell cycle through the G1 phase.
RAS Pathways

1. RAS $\rightarrow$ RAF $\rightarrow$ MEK $\rightarrow$ ERK1/2 $\rightarrow$ TFs $\rightarrow$ Cyclin D $\rightarrow$ Cell-cycle progression

2. RAS $\rightarrow$ PI3K $\rightarrow$ PIP3 $\rightarrow$ AKT $\rightarrow$ MDM2 $\rightarrow$ Apoptosis
IV. NFκB Pathway

• Important Proteins
  1. NFκB
  2. IκB
  3. IKK

• NFκB Pathway
The Proteins NFκB, IκB and IKK

- The protein complex NFκB is a transcription factor.
- Inactivated NFκB resides in the cytoplasm, bound to inhibitory proteins IκB.
- Extracellular signals (e.g., Tumor Necrosis Factor (TNF)) activate the IκB kinase (IKK).
- IKK phosphorylates IκB, leading to IκB degradation, and translocation of NFκB into the nucleus to activate the transcription of DNA.

Some specific pattern molecules, e.g. HMGB1, could also stimulate its RAGE and TLR4 to activate NFκB signaling pathway.

Modified from Wiki
NFκB Pathway

- **NFκB-IκB feedback loop:**

  Signals/Oncoproteins $\rightarrow$ IKK $\rightarrow$ IκB degrade $\rightarrow$ NFκB enter nucleus $\rightarrow$ IκB synthesis/Anti-apoptosis protein synthesis /Inflammation

- **NFκB-A20 feedback loop:**

  NFκB $\rightarrow$ A20 transcription $\rightarrow$ IKK $\rightarrow$ IκB degrade