

Gene Knockout Experiments to Quantify a G2/M Genetic Network Simulation for Mammary Cancer Susceptibility

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Abstract

A G2/M genetic network simulation is trained to reproduce published mammary cancer incidence data resulting from knockout mouse genotypes involving *ATM*, *p53*, and *BRCA1*. The genetic network is implemented using a neural network and trained using a novel knockout technique with a breeding particle swarm algorithm. We find that the gene knockouts allow the simulation to reproduce behaviour from *in vivo* mouse knockout experiments. The genetic network simulation is shown to reproduce known qualitative TSG susceptibilities resulting from *in vivo* knockouts of *ATM*, *BRCA1*, and *p53*. Two weight analyses are used to interpret the trained genetic network simulation and comparatively quantify the role of each TSG in mammary cancer susceptibility.

1 Introduction

Tumour suppressor genes (TSGs) prevent or inhibit progress through the cell cycle [5]. TSGs are integral in preventing human breast cancer and are potential diagnostic and therapeutic drug targets [19]. Animal models have shown that mutations in TSGs result in increased susceptibility to various cancers [7]. It has also been shown that endogenous mutations, caused by cellular reproduction and cellular metabolites, result in accumulating somatic mutations of TSGs over the lifetime of an individual—leading to sporadic cancers [3].

Mus musculus is the model organism of choice for studying human breast cancer. The mouse genome is well studied and known to have well conserved genes and genetic network pathways. Transgenic mice can be created with mutated or knockout TSGs leading to mammary cancers. Mouse TSG knockout models provide a measurable increase in susceptibility to mammary cancer. Unfortunately, knockout mice have only been studied since the mid-1980's and a limited number of knockout genotypes are available [7, 8]. Some TSG knockout genotypes, such as *BRCA1*^{-/-}, result in fatal embryonic development and fail to produce adult mammary cancer data [27]. Other TSG knockout genotypes (such as *p53*^{-/-}) result in other aggressive cancers (thymic lymphoma) before mammary cancer susceptibility can be measured [2]. In this paper, we use a genetic network simulation to combine the mammary cancer incidence data from several TSG knockout genotypes allowing a comparison between all knockout genotypes used for training. The resulting trained genetic network can be used to simulate fatal knockout genotypes and knockout genotypes resulting in interfering cancers.

A neural network implementation is used to implement the genetic network [25, 6, 24, 22]. Since cancer is defined as abnormal activation of the cell cycle and knockout TSGs involved in the G2M network

result in mammary carcinogenesis [7, 8], we focus on the simulating the genetic network responsible for inhibiting the G2M phase of the cell cycle [5]. The genetic network simulation is trained to reproduce mammary cancer incidence data resulting from knockout TSGs. Although knockout genotypes have been used to quantify gene interactions by Winzeler *et al.* [26] and Kaufman *et al.* [13], this simulation is novel because it uses a neural network implementation of the G2/M genetic network and knockout topologies.

Hakem and Mak [7] describe the importance of several TSGs that inhibit the G2M phase of the cell cycle. Knockout mice with a $p53^{+/-}$ genotype are described as being “predisposed” to tumourigenesis and likely to become cancerous within 9 months of age. Knockout mice with an $ATM^{+/-}$ genotype are healthy, but show increased sensitivity to ionising radiation. Whereas, knockout mice with a $BRCA1^{+/-}$ genotype show no increase in tumour incidence. We hypothesise that a genetic network simulation trained to reproduce knockout mammary cancer data will reproduce the TSG susceptibility ordering: $p53 > ATM > BRCA1$. Furthermore, we hypothesise the trained genetic network simulation will provide a quantitative measure for each TSGs contribution to mammary cancer susceptibility.

2 Genetic Networks

Figure 1: Genetic Network Diagram of G2/M checkpoint for cell cycle control. Arrows represent activation. Flat arrows indicate inhibitory interactions. Courtesy of Cell Signaling Technology.

Decades of biochemical research have resulted in genetic network diagrams, such as Figure 1, describing qualitative gene-to-gene, protein-to-gene, and protein-to-protein interactions. Genetic network diagrams show basic activating (arrows) and inhibitory (flat arrows) dependencies between genes [12, 18, 15, 11].

We model the genetic network controlling G2/M transition of the cell cycle for three reasons. By definition, carcinogenic behaviour requires a population of abnormally reproducing cells; the cell cycle is critical to this pathological state [21, 12]. Although $p53$, for example, is involved in other genetic networks leading to apoptosis, this model quantifies cell cycle inhibition resulting from $p53$ and apoptotic failure may ultimately result in abnormal activation of the cell cycle [12, 18, 15, 11]. Also, dysfunctional TSGs, such as $p53$, $BRCA1$, and ATM , are known to result in carcinogenesis by failing to inhibit G2/M phases of the cell cycle [7, 8].

Traditionally, genetic network models attempt to reproduce molecular regulatory behaviour of interactions between DNA, RNA, proteins, and small molecules [10]. Complexity challenges emerge when attempting to accurately simulate a genomic network consisting of approximately 30,000 nodes (genes) [1]. Entire molecular biology labs may spend decades focusing on interactions involving one of these nodes; it is not possible to accurately and precisely reproduce *in vivo* complexity without abstraction [4, 17]. Simpler models are required to model genetic networks. The genetic network simulation presented in this paper results from a systems biology [14] abstraction of the G2M genetic network. Interactions between genes are conserved, but limited to available *in vivo* knockout TSGs resulting in increased mammary cancer susceptibility.

3 Knockout Mice and Cumulative Tumour Incidence

Mice and humans have well-conserved genetic networks and mice are an established model organism for studying human mammary carcinogenesis [7]. Knockout mice have one or both germ line copies of a

particular allele deleted. Mice with knockout TSGs often exhibit increased susceptibility to carcinogenesis [9, 7, 8]. Cumulative tumour incidence graphs describe onset of carcinogenesis in a population over time and are often used to show cancerous susceptibility of knockout mice.

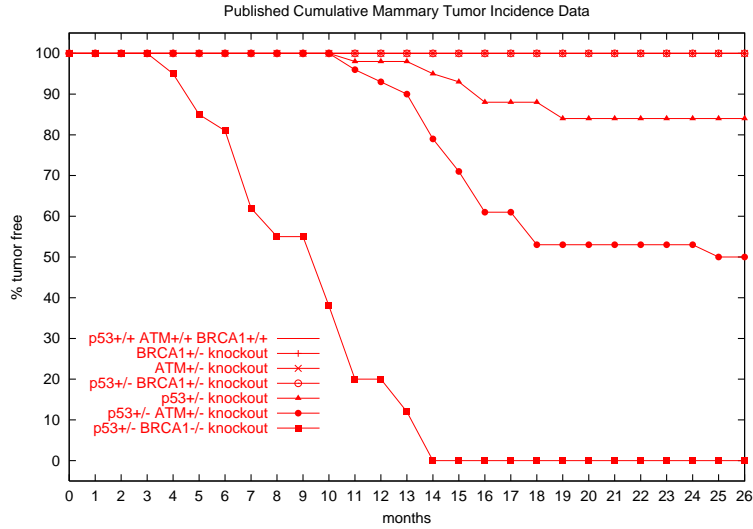


Figure 2: Cumulative mammary tumour incidence produced from $p53^{+/-}ATM^{+/-}$ (Umesako *et al.*) and $p53^{+/-}BRCA1^{-/-}$ (Xu *et al.*) knockout genotypes. Note control genotype and several knockout genotypes remain 100% tumour free and as a result overlap.

Figure 2 shows data from two publications using TSG knockout genotypes to study mammary carcinogenesis. Umesako *et al.* used mice with knockouts involving p53 and ATM [23]. Xu *et al.* used mice with knockouts involving p53 and BRCA1 [27]. Both papers presented data for the $p53^{+/-}$ genotype; strain susceptibility differences were accounted for by combining tumour incidence data for both strains. TSG knockout combinations resulted in increased susceptibility to mammary carcinogenesis.

Increased mammary cancer susceptibility resulting from these knockout TSGs to mammary carcinogenesis is not directly comparable. For example, $p53^{+/-}ATM^{+/-}$ mice have one deleted *ATM* allele, whereas $p53^{+/-}BRCA1^{-/-}$ mice have two *BRCA1* alleles deleted. Although, the tumour incidence data shown in Figure 2 shows a pronounced difference in susceptibility, one dataset results from a one allele knockout whereas the other results from a two allele knockout. Since the genetic network simulation is trained with both data sets, a comparison between *ATM* and *BRCA1* knockouts is quantified.

4 Model Design

4.1 Neural Network Implementation

A neural network design is used to simulate the G2/M genetic network for several reasons. Neural networks are an established computational paradigm [16] functionally similar to genetic networks. Neural networks consist of a group of nodes with weighted interactions which interpret input signals and produce a corresponding output.

Neural networks have been used to model protein/genetic networks. Jiri Vohradsky published a neural network model of genetic network control for lysis/lysogeny in λ bacteriophage. Vohradsky's model was able to reproduce experimental data for the six gene network and elicit a realistic phage transition from a lysogenic state to a lytic state [24]. Welch *et al.* used a neural network to model flowering time control in

Arabidopsis thaliana. An eight gene network was trained to realistically react to external environmental factors such as day length and days after planting to initiate flowering [25].

$$output_n = f \left(\sum_{i=1}^m (wt_i \times input_i) + wt_{min} \right) \quad (1)$$

For this implementation, each node output ($output_n$) results from the sum of input signals ($input_i$) multiplied by a weight (wt_i). Weights are positive for activation and negative for inhibition. A minimal weight (wt_{min}) provides node output when all input signals are zero. Resulting output becomes the input for following nodes in the network.

Figure 3 shows a simple three node example where node C 's output ($output_C$) is dependent on inputs from nodes A and B . Note that C 's input signals are dependent on the output of nodes A and B .

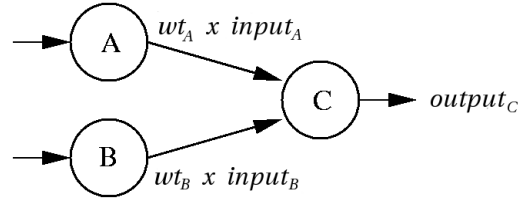


Figure 3: A three-node network example.

Equation 1 is then modified to handle diploidy knockout genotypes and mutation. In Equation 2, a knockout variable ($knock$) is toggled from one to zero for allele deletion. A cumulative stochastic mutation variable (mut) decreasing from one to zero represents genetic mutations causing the gene to degrade over time. Two copies (j) of mutation and knockout variables represent two allele copies of the gene. Gene degradation (mut) and dual alleles (j) are not present in the Vohradsky and Welch *et al.* models. These extensions (mut and j) are novel to a neural network implementation of a genetic network.

$$output_n = f \left(\sum_{j=1}^2 knock_{n,j} \times mut_{n,j} \times \left\{ \sum_{i=1}^m (wt_i \times input_i) + wt_{min} \right\} \right) \quad (2)$$

An activation function, $f(x)$, is applied to each node resulting in sigmoidal non-linear behaviour associated with neural networks. For computational tractability, we use a simple linear activation function over a bounded region to simulate a non-linear response.

$$f(x) = \begin{cases} 0 & : x < 0 \\ x & : 0 < x < 1 \\ 1 & : x > 1 \end{cases} \quad (3)$$

4.2 Knockout Training

Overlapping gene functionality is used to apply the knockout tumour incidence data to the G2M genetic network. For example, without a knockout, the mice remain tumour free. Whereas a knockout genotype, such as $p53^{+/-}BRCA1^{-/-}$, results in increased tumour incidence. The difference is one copy of the p53 allele and both copies of the BRCA1 allele. All other TSGs are intact; the resulting susceptibility can be attributed to the remaining intact genome.

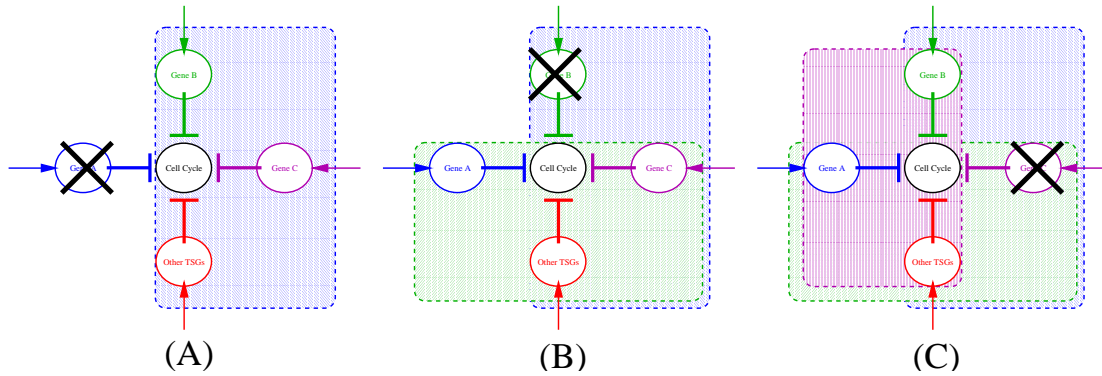


Figure 4: Illustration of training data overlap from the knockout gene complements.

If one TSG is knocked out, Figure 4(A), the resulting carcinogenic phenotype is due to the remaining genome’s functionality. Tumour incidence data from knockout mice provide a metric for the remaining genome’s susceptibility to mammary cancer. Figures 4(B) and 4(C) illustrate the overlap of data for three possible TSG knockout genotypes.

Each knockout produces data complementary to the specific knockout gene. To train a genetic network with this data, the network’s topology is simplified to the knockout gene(s) and the complement of the knockout gene(s). Without this simplification, all of the genomic network would need to be simulated and there would be too many attributable genes; the interactions would not be accurate or consistent. Network topology is limited to the specific knockout genes and “other TSGs” remaining intact that inhibit the G2M phase of the cell cycle. As a result, the G2M genetic network can be simplified to a network (Figure 5) involving p53, ATM, BRCA1, and “other TSGs.”

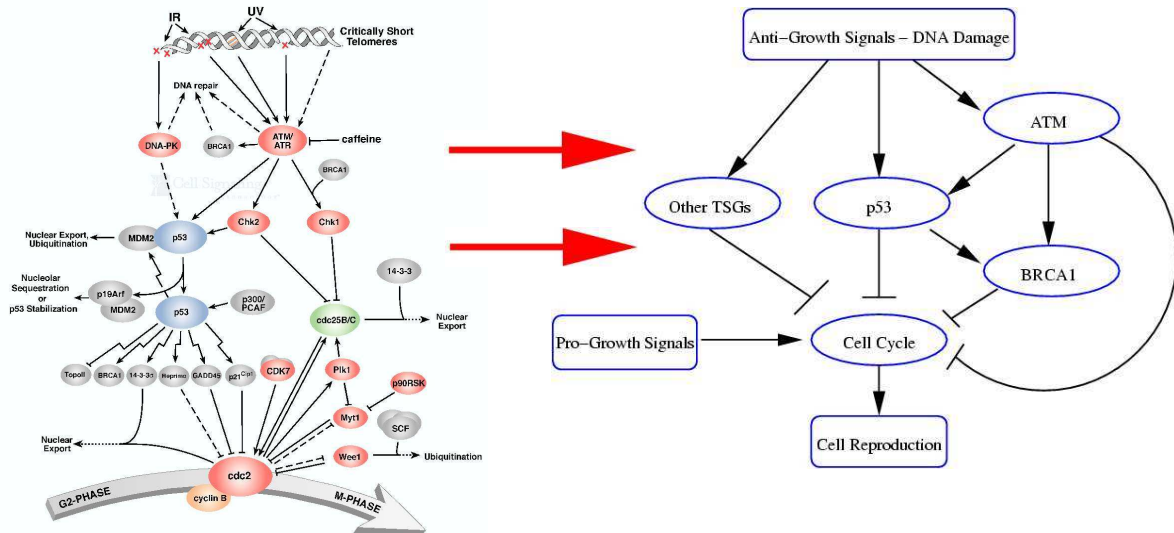


Figure 5: G2/M cell cycle genetic network (left) and genetic network abstraction (right). The abstraction conserves activation and inhibition signals from the more detailed G2/M cell cycle genetic network.

Training the simulated genetic network involves assigning the correct weights, w_{ij} , to each node. Weights of the simulated genetic network are trained using a Breeding Particle Swarm Algorithm (BPSA) described by Settles and Soule[20]. A **fitness value** is assigned to each set of candidate weights, so the BPSA can effectively search for optimal weights. After candidate weights are assigned to the network, a

fitness value is determined based on two measures: Feasibility and Mouse Simulations.

4.3 Feasibility

This measurement indicates correct interpretation of input signals. Input signals are classified as pro-growth and anti-growth. A pro-growth signal, such as growth factor signaling, should turn reproduction on. Anti-growth, such as DNA damage or apoptotic signaling, should prevent a cell from reproducing. The feasibility measurement maintains basic realistic network functionality. Given pro-growth signals, a realistic genetic network results in cell cycle activation; anti-growth signals inhibit the cell cycle.

Input Signals		Output Signals
Pro-Growth	Anti-Growth	Reproduction
0	0	1
0	1	0
1	0	1
1	1	0

Table 1: Feasibility scenarios used to judge basic network functionality. 0 = off signal, 1 = on signal.

4.4 Mouse Simulations

TSGs in the network are disabled using *knock* variables (from Eq. 2) to reflect the mouse knockout genotypes responsible for tumour incidence data. Cumulative stochastic mutation (*mut*) causes each gene (*n*) to deteriorate from one to zero. Since tumour incidence in Figure 6 approximates a cumulative normal distribution, Equation 4 shows the cumulative normal distribution used to degrade the genetic network. Varying mean and variance of the normal distribution from .02 and .01 respectively, still allows the simulation to be trained—these values are arbitrary.

$$mut_{n,j} = mut_{n,j} - Norm(.02, .01) \quad (4)$$

Each mouse simulation accumulates mutation at 26 discrete time points representing 26 months—analogue to somatic mutations accumulating throughout the 26 months the *in vivo* knockout mice were observed. The mutated network has a cancerous state when the network output, Reproduction in Table 1, is greater for all Input Signal combinations than the normal network output defined in Table 1. If the mutated network’s output indicates a cancerous state, the time point is recorded. This time point is analogue to time-to-tumour data of a laboratory knockout mouse.

100 instances of the network simulate a population of one hundred mice. Simulated cumulative tumour incidence is compared to *in vivo* published cumulative incidence data using sum of squares difference at each time point. The BPSA optimises network weights to minimise the difference between the simulated and *in vivo* data.

Figure 6 shows an example of a well-trained network fitting data from three knockout genotypes [23, 27]. The simulated and *in vivo* $p53^{+/-}BRCA1^{-/-}$ knockout genotype results in earlier and more severe tumourous growth. Whereas the simulated and *in vivo* $p53^{+/-}ATM^{+/-}$ knockout genotype results in tumour onset in older mice with less frequency. Finally, the $p53^{+/-}$ knockout genotype results in fewer tumourous mice with the least frequency.

5 Simulation Analysis

Based on node behaviour (Section 4.1) and mouse simulation training (Section 4.4), two measures result: I/O Contribution and Robustness.

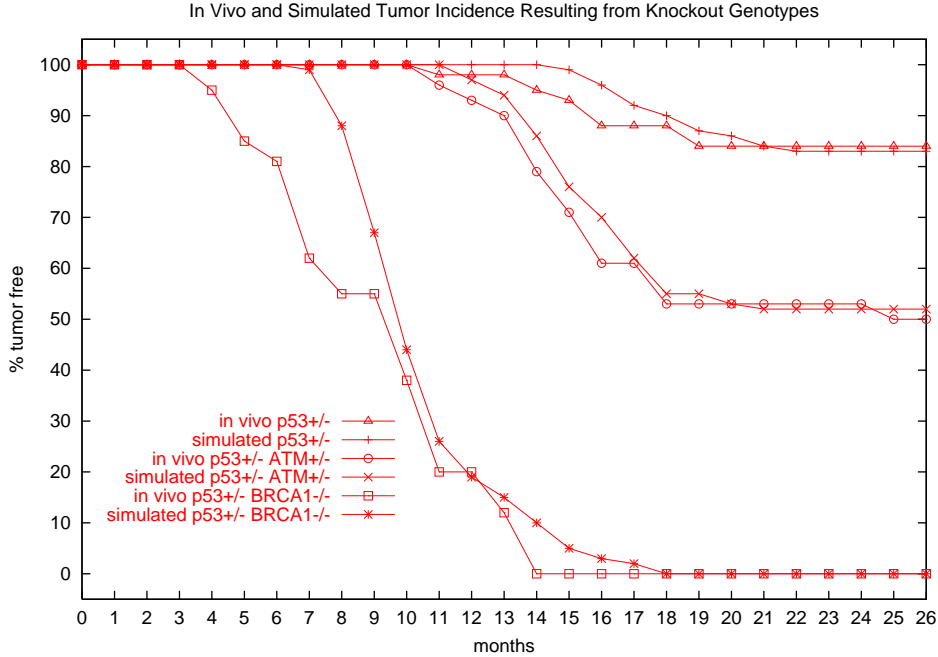


Figure 6: *In vivo* and simulated time series data comparison. Genetic network simulation results are from a single network. Cumulative mammary tumour incidence produced from *in vivo* and simulated $p53^{+/-}ATM^{+/-}$, $p53^{+/-}BRCA1^{-/-}$, and $p53^{+/-}$ knockouts.

360 networks were trained; each network was trained using the same knockout data. BPSA training lasted for 10,500 generations taking approximately 850 minutes on a Beowulf cluster with dual xeon processors. Since the BPSA is stochastic, fitnesses varied. Removing 1/3 of the least fit runs resulted in an average fitness of .04493 for the remaining 240 networks and prevented unrealistic networks from possibly corrupting the gene network analyses.

5.1 I/O Contribution

Given the knockout mouse data used to train the genetic network simulation, which TSGs were most important to inhibiting the G2/M phase of the cell cycle? I/O Contribution measures a gene’s importance to the genetic network’s output. I/O contribution is measured by knocking out both copies of gene ($knockout_n$) and comparing network output to the output of the intact network ($intact_n$). Mutation (mut) is fixed to .01 so that the activation function (Equation 3) does not clip signals resulting from larger weights. The change in network output due to gene n is specified as io_n in Equation 5. Nodes with higher io_n scores have a higher contribution to the correct functionality of the network.

$$io_n = |network(knockout_n) - network(intact_n)| \quad (5)$$

Figure 7 shows average io_n scores for 240 networks trained with Feasibility (Section 4.3) and Mouse Simulations (Section 4.4) and 240 control networks trained only with Feasibility. $p53$ has the greatest effect on genetic network output, followed by $BRCA1$ and ATM . Note the overlapping error bars of the io scores of ATM and $p53$ in control networks. However, genetic network simulations trained with knockout mouse data show io_n scores differ significantly for the 95% confidence interval. Signal from the knockout mouse simulation training resulting in an io_n score ordering of $p53 > ATM > BRCA1$. This ordering is consistent with our hypothesis mentioned in the introduction.

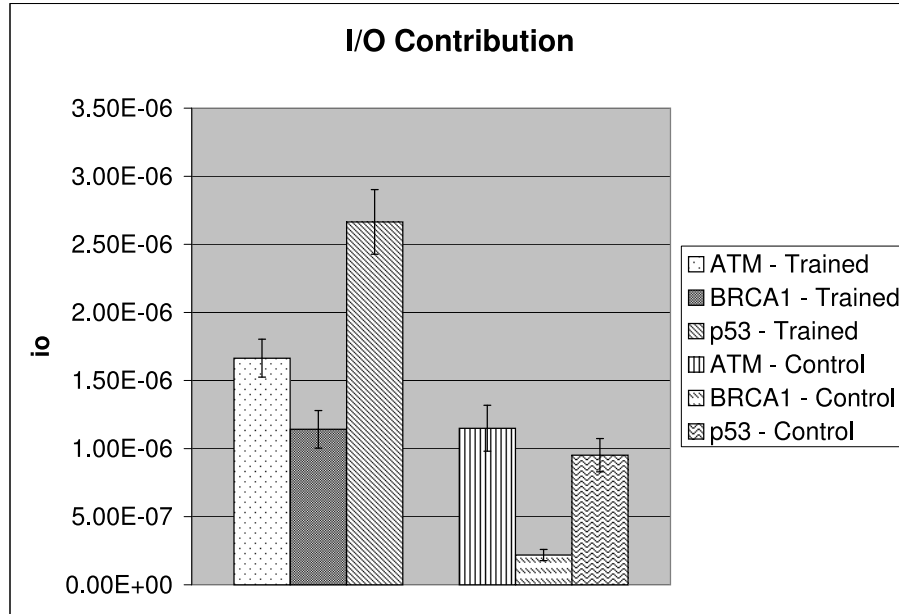


Figure 7: Average I/O contribution for 240 networks trained with *ATM*, *BRCA1* and *p53* tumour incidence (left) and 240 control networks trained without mouse simulations described in Section 4.4. Error bars indicate 95% confidence interval.

5.2 Robustness

Robustness, in this genetic network simulation, measures the ability of a gene, in the network, to function despite accumulating mutations described in Equation 4. A score for robustness (*robust*) is attained by adding together all inputs weights (wt_i) for node (n). Equation 2 shows as mut_n decreases with time, larger weights are necessary to prevent gene n from mis-reading input signals (*input*). Smaller weights are assigned to genes more sensitive to mut_n and more likely to dysfunction. A larger *robust* score indicates the gene is more resistant to simulated mutation.

$$robust_n = \sum_{i=1}^m |wt_i| + wt_{min} \quad (6)$$

Figure 8 shows average *robust* scores for 240 trained networks (left) and 240 control networks (right). The *BRCA1* node is assigned larger input weights resulting in a higher average robust score; the genetic network simulation trained with knockout mouse data indicates that *BRCA1* is more resilient to simulated mutations. *ATM* is assigned a much lower average score—indicating that it is more likely to dysfunction and result in mammary carcinogenesis according to the genetic network simulation.

Note the overlap of error bars and differences in magnitude of the control networks trained without knockout data. Markedly different behaviour between trained and control robustness scoring indicates that training with tumour incidence data is informing the genetic network simulation.

6 Discussion

We have presented a genetic network simulation which is trained to reproduce *in vivo* knockout mouse tumour incidence data. The simulation reproduces qualitative mammary cancer susceptibility resulting

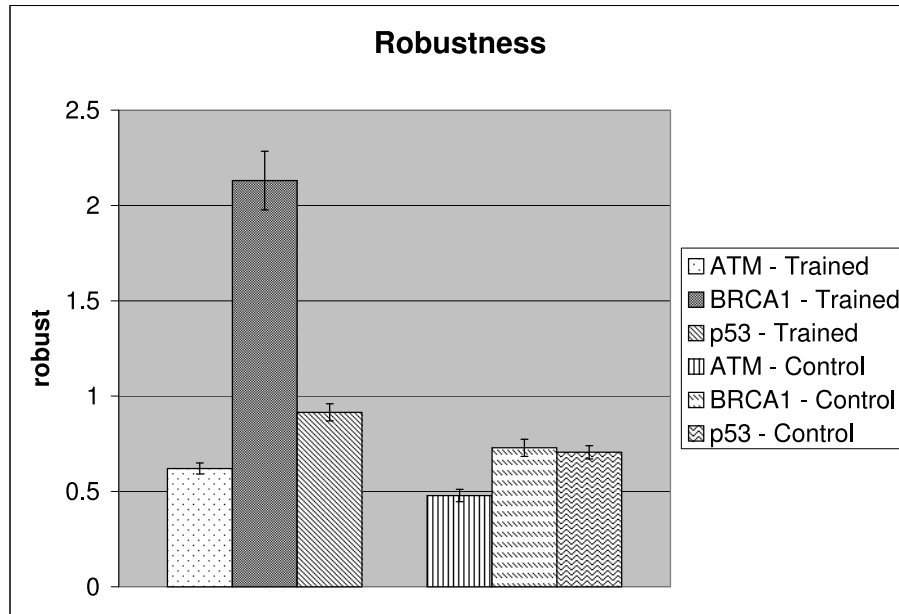


Figure 8: Average *robust* scores for 240 networks trained with *ATM*, *BRCA1* and *p53* knockout data (left) and 240 networks trained without knockout data (right). Error bars indicate 95% confidence interval.

from TSG knockouts. Since mammary cancer susceptibility of a knockout genotype results from the remaining intact genome, multiple TSG knockouts provide overlapping data regarding the remaining intact TSGs.

Based on *in vivo* data, this genetic network simulation allows a quantified comparison between TSG knockout genes: *ATM*, *BRCA1* and *p53*. *I/O contribution* analysis indicates that *p53* is most relevant to inhibiting the cell cycle whereas *BRCA1* is the less important to mouse mammary cancer susceptibility. The qualitative ordering of $p53 > ATM > BRCA1$ is consistent with mouse mammary cancer literature. This simulation provides a quantitative model for increased mammary cancer susceptibility due to TSG dysfunction.

Since this genetic network simulation involves degrading the network via accumulating somatic mutations, a measure for genetic robustness is presented and used to analyse the trained simulation. It is predicted that the *BRCA1* TSG is most resistant to somatic mutation over the lifetime of *Mus musculus*. *ATM* has the lowest *robust* score indicating that it is more likely to dysfunction.

We acknowledge that carcinogenesis may result from other forms of genetic dysfunction, our model simulates genetic susceptibility to carcinogenesis in the context of somatic mutations. We plan to extend the simulated genetic network and trained data as more knockout mouse experiments are published.

Researchers need a preliminary understanding of which genes to target before spending valuable time and resources investigating a gene that doesn't contribute to a particular form of carcinogenesis. Although there is no substitute for molecular bioscientific investigation, this genetic network simulation can be used as a guide to elicit hypotheses regarding cancer genetics and susceptibility.

Acknowledgement

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