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Chapter 1

Introduction

The thesis presents research into genetic interactions based on genomics data and bioinformatics approaches. This chapter introduces the recent developments in genomics and bioinformatics, particularly in their application to cancer research. Synthetic lethal interactions are a long standing area of research in genetics in both model organisms and cancer biology. Various reasons why these interactions are of interest in fundamental and translational biology will be outlined but first these and similar interactions will be defined. A bioinformatics approach to synthetic lethal interactions enables much wider exploration of the inter-connected nature of genes and proteins within a cancer cell than previous candidate-based approaches. An alternative approach is experimental screening which will be presented and contrasted with bioinformatics approaches in more detail. An emerging application of synthetic lethality is the design of treatments with specificity against loss of function mutations in tumour suppressor genes. E-cadherin (encoded by *CDH1*) is a prime example of this which will be the focus of the analysis in this thesis and as such the role of this gene in cellular and cancer biology will be briefly reviewed.

1.1 Cancer Research in the Post-Genomic Era

Genomics technologies have the potential to vastly impact upon various areas including health and cancer medicine. Considering the progress in recent genomics research, it could soon impact greatly upon clinical and wider applications of genetics either directly or by enabling more focused genetics research from candidates selected from genomics or bioinformatics analysis. The completion of the draft Human Genome (Lander *et al.*, 2001) marks a major accomplishment in genetics research and raises

new challenges to utilise this genomic scale information effectively. Technologies in this area have rapidly developed since completion of the human genome project and many global large-scale projects have to expanded upon the human genome, to populations (1000 Genomes, 2010), to cancers (Dickson, 1999; Zhang *et al.*, 2011), and to deeper functional understanding (ENCODE, 2004; Kawai *et al.*, 2001). However, impact on the clinic has been slower than initially anticipated following the completion of the “draft” genome with genomics technologies yet to become widely adopted in healthcare and oncology. Here we outline the genomics technologies and bioinformatics approaches which have led to availability of genomics data and techniques used in this thesis and potential for applications in cancer research or the clinic in the future.

1.1.1 Cancer as a Global Health Concern

- describe/define
- incidence / prevalence / morality (global)
- incidence / prevalence / morality (NZ)
- Current diagnostics: largely pathological
- Current treatment: surgery, radiation, chemotherapy

1.1.1.1 Genetics and Molecular Biology in Cancers

- Genetics and Heredity of Cancers
- Hallmarks of Cancer
- Intrinsic subtyping and Pan cancer approaches

1.1.2 The Human Genome Revolution

The advent of the Human Genome sequence has transformed genetics research (Lander *et al.*, 2001). Systematic, unbiased studies across all of the genes in the genome are viable in unprecedented ways. The successful undertaking of such an international scientific megaproject has set an example for numerous initiatives to follow, including many genomics investigations expanding to species, to the functional, or to the population level. These projects serve are excellent resource for genetics research globally, particularly for cancers where it has been widely applied to different tissues across

molecular profiles. Genome sequencing technologies continue to improve, drop in price, and become feasible in more research and for clinical applications.

1.1.2.1 The First Human Genome Sequence

The first human genome is a good example of a large-scale genomics project for its success as an international collaboration and releasing their data as a resource for the wider scientific community. This particular project generated significant public interest due to it being a landmark achievement, the first of its scale, and some controversial findings. Namely, the number of genes discovered (particularly those specific to vertebrates) was much lower than most estimates of a genome of its size and the number of repetitious transposon elements was very high. Even the figure of 30-40,000 genes given by the original publication is now widely viewed as an overestimate.

Accounting for the “complexity” encoded by the human genome with so few genes has led to investigations into molecular function, expression profiling, and population variation. When publishing the genome, the authors conceded that genomic information alone is not biological understanding and that many investigations remain to be done, with their main goal being to share the raw genome data so that it was available for further inquiry rather than interpreting it themselves. While genomics technologies and genomics projects have flourished since then, the need in turn for systematic means of interpreting data of such scale and for the interdisciplinary expertise to do so has only grown.

1.1.2.2 Expectations of Genomics

Genomics has stimulated investigations into many of these previously largely explored areas of functional genetics and thus been of immense value in genetics research, attracting high expectations for further applications. Genomics research has become anticipated for its potential for widespread applications in healthcare, agriculture, ecology, conservation, and evolutionary biology.

An area of particularly high interest for the clinical impact of genomics is oncology, with potential application across cancer diagnostics, prognosis, management, and developing treatment. Cancers are diseases characterised by uncontrolled cell growth, often driven by genetic mutation or dysregulated gene expression. However, as with many areas of genomics, direct impact of genomics on the clinic has been limited compared to initial expectations following the publication of the human genome and compared with widespread adoption in cancer research.

1.1.3 Technologies to Enable Genetics Research

1.1.3.1 DNA Sequencing and Genotyping Technologies

Genotyping was once commonly performed on variable regions of the genome with restriction fragment length polymorphisms (RFLP) or repetitious microsatellite regions. These exploited sequence variation at target sites of restriction enzymes or measured the length of repetitious regions, using polymerase chain reaction (PCR), restriction enzymes, and gel electrophoresis to measure DNA genotypes at particular sites. This is laborious and limited to well characterised variable regions of the genome, generally genes or nearby marker regions.

The Sanger (dideoxy) chain termination method enabled DNA sequencing and genotyping at a wider scale than previously possible. This quickly became more widely adopted over the Maxam-Gillert sequencing by degradation method developed around the same time due to the technique being less technically difficult and requiring less radioactive and toxic reactants, which have since been replaced by fluorescent dyes. Another advantage of the Sanger methodology is the relatively long read length (particularly compared to early versions of more recent technologies), with read lengths of 500-700 base pairs accurately sequenced in most applications, usually following targeted amplification with PCR. Sanger sequencing by gel electrophoresis takes around 6-8 hours and has been further refined with the “capillary” approach to 1-3 hours and requiring less input DNA and reactants. The capillary approach has been scaled up to run in parallel from a 96 well plate, at 166 kilobases per hour. The 96 well parallel capillary method was one of the main innovations which made the first Human Genome Project feasible and was used throughout. Due to the quality of the Sanger sequence reads and low cost, it is still widely used in smaller scale applications, clinical testing, and as a “gold standard” to validate the findings of newer approaches.

1.1.3.2 Microarrays and Quantitative Technologies

Real-time or quantitative PCR (qPCR) is another adaptation of genetic technologies to quantitatively study nucleic acids, often reverse transcribed “cDNA” or messenger “mRNA” to measure (relative) gene expression or transcript abundance. While numerous quality control measures are required to correctly interpret a qPCR experiment, these have similarly become widely adopted as are still used for smaller scale experiments and as a “gold standard” for measuring gene expression (Adamski *et al.*, 2014). This also represents a shift in the application of PCR and sequencing technology, where

the primary interest is quantifying the amount of input material (by the rate of amplification to a certain level) rather than the qualitative nature of the sequence itself. The more recent technologies of microarrays and RNA-Seq have similarly embraced this application to quantify DNA copy number, RNA expression, and DNA methylation levels. Due to results of comparable or arguably better quality from these newer technologies (Robin *et al.*, 2016; Beck *et al.*, 2016; McCourt *et al.*, 2013; Git *et al.*, 2010), this “gold standard” status has started to come under scrutiny.

Microarrays represent a truly high-throughput molecular technique, reducing the cost, time, and labour required to study molecular factors such as genotype, expression, or methylation across many genes, making it feasible to do so over a statistically meaningful number of samples. Microarrays are manufactured with probes which measure binding of particular nucleotide sequences to either quantitatively detect the presence of a sequence such as a single nucleotide polymorphism (SNP) or quantify DNA copy number, gene expression, or DNA CpG methylation. Microarray technologies have popularised “genome scale” studies of genetic variation and expression.

In addition to being more versatile and higher-throughput than PCR based techniques, microarrays are considered cost-effective, particularly when scaled up to large number of probes. They are also available with established gene panels or customised probes from a number of commercial manufacturers. These remained popular during the introduction of newer technologies due to reliability and this relatively lower cost, especially in large-scale projects involving many samples. However, microarrays have issues with signal-to-noise ratio with both sensitivity to low nucleic acid abundance and “saturation” of probes at high abundance and require more starting material than qPCR. Thus qPCR is still used for many small gene panel studies.

1.1.3.3 Massively Parallel “Next Generation” Sequencing

Similar to microarrays, the introduction massively parallel sequencing technologies have further expanded the availability of high-throughput molecular studies to researchers, with corresponding availability of genomics data from these studies. This “Next-Generation Sequencing” (NGS) expands not only gene expression studies (compared to microarrays) but extends to genome sequencing *de novo* for previously unknown genome and transcriptome sequences at an unprecedented scale. This has been a particularly important technological revolution in genomics, as the cost and time of genome sequencing has dropped dramatically and enabled sequencing projects of far more samples and applications beyond the Human Genome Project. Particularly,

when dealing with variants in a species with an existing reference sequence such as humans, where the computational cost of mapping to a reference over a genome assembly. However, the cost of sequencing (RNA-Seq) for gene expression or DNA methylation studies is still considerably higher than a microarray study (limiting feasible sample sizes).

Compared with arrays, NGS studies have additional challenges, particularly with large data and compute requirements to handle the raw output data. Compared the the established methods to analyse microarray data, handling NGS data can be more technically difficult. While methods developed for analysing microarray data can be repurposed for sequence analysis in many cases, more bioinformatics expertise is required particularly to handle the raw read data and changing approaches for various changes in sequencing technologies. One of the main computational challenges is the assembly reads or mapping to a reference genome due to the inherently small reads of most NGS technologies compared to the Sanger methodology. Furthermore, there are fewer software releases and best practices established specifically RNA-Seq data, thus many analyses are still conducted with customised analysis approaches and command-line tools. Compared to existing graphical tools or pipelines for microarray analysis, this is a more active technology for bioinformatics research with many applications of genomics data have yet to be explored.

However, the methodology itself has challenges with the sample preparation, requiring a relatively high quantity of input material and “contamination” with over abundant ribosomal rRNA taking up the majority of the sequencing if not purified correctly. This abundance of rRNA is a particularly important issue in microarray and RNA experiments in Eukaryotes where it is commonplace target the mRNA by binding to the poly-A tail (RNA-Seq) or 5’ cap (CAGE-Seq). However, this has the potential to exclude microRNAs (miRNA) and long non-coding RNAs (lncRNA) of interest unless the sample is prepared specifically to study these. Similarly capturing a subsection of the genome for exome analysis or reduced representation bisulfite sequencing (RRBS), focuses on sequencing DNA sequences and methylation levels of CpG sites near known genes to reduce cost, noise, and incidental findings.

In many cases, the benefits of NGS technologies over microarrays still outweigh the additional cost. NGS is highly adaptable to different applications: DNA sequencing (whole genome or exome), DNA methylation (bisulfite-Seq), RNA-Seq, miRNAs, lncRNA, or chromatin immunoprecipitation (CHIP-Seq). NGS scales to all genes and beyond for these molecular applications without having to design new probes as re-

quired for a microarray. Thus NGS technologies are not limited to genes already characterised sequence or functions, do not need to be updated with new probes for each genome annotation release, and do not require a reference genome at all for new species. A “transcriptome” can be assembled *de novo* for an expression study in any organism.

NGS technologies also have the advantage of greater potential accuracy and sensitivity than microarrays, depending on the sequencing depth or “coverage”, theoretically sensitive down to the exact number of molecules for each transcript. NGS experiments are regarded as “reproducible” with no need for technical replicates, although these are still performed for a subset of samples in many projects for quality assurance purposes. NGS has a wider dynamic range than microarrays and is able to detect SNPS, InDels, and splice variants in addition to quantifying DNA copy number or transcript abundance.

1.1.3.3.1 Molecular Profiles with Genomics Technology

[More Info here]

Reverse transcribed RNAs are a common such adaptation, employing RNA-Seq to the transcriptome. This is utilised to be quantify the levels of RNA and identify which regions of DNA are expressed. Similar bisulfite treatment converts cytosine residues to uracil (sequenced as thymidine), sparing methylated cytosine enabling it to be distinguished with bisulfite-Seq for high-throughput detection of the notable epigenetic mark and generating an epigenome. High-throughput gel and mass spectrometry techniques have been employed to proteins and metabolites to generate the proteome and metabolome respectively. In this way, so called “omics” profiles across a wide range of biomolecules in a cell are produced in many experimental laboratories. Such genomics technologies have since been applied to single cell isolates and to detect traces of foetal or tumour molecules in blood or urine.

1.1.3.3.2 Established Sequencing Technologies

454 sequencing (acquired by Roche) commercially released from 2005 to 2013 was the first NGS technology, generating a vast 1 million reads per day or 400-600Mbp in a 10 hour run. This technology used the “pyrosequencing” method of sequencing by synthesis, detecting phosphates released when a compatible nucleotide reacts and extends the DNA synthesis of a complementary strand. This technology popularised NGS with the first complete genome from a single individual (Wadman and Watson,

2008; Wheeler *et al.*, 2008) and Neanderthal ancient DNA studies (Noonan *et al.*, 2006; Green *et al.*, 2009). While this technology was capable of reads up to 1kb, reads of 400-500bp were more typical and the technology had difficulties with accurately processing runs of repeated bases. These are still relatively long reads for an NGS technology but it has been discontinued due to competing short read technologies being more cost-effective with lower running costs.

SOLiD sequencing (acquired by Life Technologies and then Thermo Fisher) released in 2006 employed a vastly different approach to NGS, using labelled dinucleotide pairs for “sequencing by ligation” to produce a highly accurate sequence (99.94%) with built-in error correction by sequencing two reading frames and is unaffected by consecutive bases. This technology is also high-throughput, producing 1200-1400 million reads (66-120Gbp) in a 7-14 day run. However, SOLiD sequencing does not cope well with palindromic sequences and SOLiD reads are very short only 35bp, making it more difficult to assemble them.

Illumina sequencing (developed by Solexa and later acquired by Illumina) was also released in 2006. It utilises reversible terminating dyes to sequence by synthesis with a lower accuracy (98%) and read lengths of 150-250bp. Illumina more than makes up for relatively short reads (along with improving the read length of the technology) and low accuracy with high-throughput and cost effectiveness, with a Hi-Seq 2500 platform producing up to 3 billion reads (600Gbp) in a 3-10 days run. Illumina has further reduced the cost of sequencing with the economies of scale with the HiSeq 10X claiming to produce a human genome for less than US\$1000, the first platform to achieve this long-standing goal in genomics. The high-throughput of Illumina sequencing also makes deep sequencing for high coverage, high quality consensus reads, and sensitive RNA-Seq experiments feasible. Illumina sequencing now has a dominating market share of the NGS technologies.

1.1.3.3.3 Emerging Sequencing Technologies

Ion Torrent (also acquired by Life Technologies) released in 2010 employs “sequencing by synthesis” but in a drastically different way with ion semiconductor sequencing, detecting H^+ ions released when bases during DNA synthesis. Without the use of optical detection, the Ion Torrent system is compact offering rapid, cost-effective sequencing with the potential to scale with the future development of silicon semiconductors with have historically doubled in density every 2 years (Moore’s Law). It is capable of reads of 100-200bp in only an hour (as fast as 4 seconds per base) and up to 400bp in a

2 hour run with an accuracy of 99.6% (dropping to 98% for consecutive sequences of 5 bases). While fast, cost effective, and accurate, Ion Torrent has short reads and modest throughput (depending on the platform 100Mbps to 32Gbps) compared to other sequencing technologies.

Pacific Biosciences (PacBio) released the RS and RS II platforms in 2010 and 2011 to make up for the short reads in NGS technologies with the single molecule real time (SMRT) approach capable of long read lengths, averaging between 2.5-7kb and up to 80kb. The PacBio methodology traps each molecule in a zero mode waveguide (ZMW) and sequences it in real time. The RS II has 150,000 ZMW and an output of 500Mbps-1Gbps per SMRT cell (doubling that of the RS), with the capacity to run up to 16 concurrently for 0.5-6 hours. While the single molecule sequencing approach has strengths in sensitivity and potential to detect 3D structures, such as G-quadruplexes, this has the drawback of slowing down the sequencing and reducing the throughput of the platform. Another issue is sequence quality with the raw data as poor as 20-30%. However, PacBio recommends specific software to assemble as consensus with 99.999% for sequences with over 20x coverage, regardless of sequence repeats or GC composition. Despite concerns over data quality and higher cost than other approaches, the long reads are appealing for genome assembly and in many genome studies combine PacBio reads with more accurate short read technologies. However, due to the poor separate quality of reads this technology may not be appropriate for RNA-Seq studies, while it does have the potential for high sensitivity and detecting alternative splicing were it be improved. PacBio has recently released the Sequel (2016) system, increasing the throughput of the SMRT Cells 7x to 1 million ZMW holes with an output of 5-10Gb for each of 16 SMRT cells.

Nanopore sequencing is another technology capable of long reads in real time and direct single molecule sequencing, avoiding amplification bias, detecting modified bases and directly sequencing RNA molecules. This also reduces laboratory preparation times. Nanopores work by measuring the ion current through a pore in a electrically insulating membrane as a nucleic moves through it. Oxford Nanopore has been developing this technology since 2005, launching the MinION in 2014 which employs biological nanopores: a transmembrane protein through which DNA or RNA passes, blocking ion current differently for each base. Each pore sequences in real time, capable of sequencing 450bp per second. However, there are quality issues with each individual read with quality estimates varying between 87-98%, with improvements to the quality of detection accounting for significant delays in the release of this technology. The Min-

ION makes up for this is a capacity for extremely long reads, averaging 5.4kbp (Hayden, 2014) up to a maximum of 200Kbp and being a portable platform with very few overhead costs. While the MinION is limited in scale with only one flow cell of 512 pores (5-10Gbp), the PromethION being released in early access in 2016 scales this technology with flow cells of 3000 pores and the capacity to run 48 (up to 4 samples each) in parallel for 144,000 long reads with a versatile, modular system including built-in computing resources. One of the main issues with Oxford Nanopore systems is accuracy, with the manufacturer suggesting the use of consensus sequences for higher accuracy as PacBio does. The main source of this pore accuracy is the width of biological pores resulting in several bases being in the pore at any one time, inferring the sequence from the ion currents of each respective combination of bases and distinguishing them is a major technical challenge.

Quantum Biosystems in Japan is developing a synthetic nanopore system to address this issue. While the technology is still in development, it has the potential to produce similarly long reads, with a high-throughput, low running cost, and rapid run time. The technical challenges to develop a nanotechnology capable of this are immense but such developments serve as but one of example of how sequencing technologies may continue to improve, becoming more feasible for a wider variety of applications.

Due to such benefits of sequencing over previous technologies (and their continued refinement), this thesis has focused on RNA-Seq data in contrast to prior studies on microarray data. RNA-Seq data is widely available as a resource from large-scale cancer genomics projects and methods to make inferences from RNA-Seq experiments could feasibly be applied to many other studies based on these current (or similar future) technologies.

1.1.3.4 Bioinformatics as Interdisciplinary Genomic Analysis

Genomics technologies have given rise to data at a scale previously rarely encountered in molecular biology, making inference with conventional techniques difficult. Computational, Mathematical, and Statistical skills are required to handle this data effectively, in addition to biological background to frame and interpret research questions. Drawing upon these disciplines to handle biological data has become the field of “Bioinformatics”, focusing specifically on making inferences from genomics and high-throughput molecular data or developing the tools to do so. This contrasts with the existing fields of “theoretical“ or “computational biology“ which existed prior to genomics data, focusing on modelling and simulating aspects of biology without necessarily addressing

the genomics data or detecting the phenomena in nature, extending beyond genetics to cell modelling, neuroscience, cancer development, ecology, and evolution.

In practice, many researchers identify with both bioinformatics and computational biology, or draw upon the findings and methods of the other field. This thesis uses many approaches in bioinformatics to biological research questions and established mathematical or bioinformatics resources.

Gene expression analysis is the focus of many bioinformatics research groups, drawing upon statistical approaches to appropriately handle microarray and RNA-Seq data along with making biological inferences from a large number of statistical tests. This presents various challenges from normalising sample data and accounting for batch effects to developing or applying statistical tests tailored to biological hypotheses and testing them at a genome-wide scale, generally across thousands of genes. There are numerous approaches for dealing with these challenges, some of which will be described in chapter ??.

1.1.3.5 Gene Expression Studies with Microarrays

Text

1.1.4 Follow-up Large-Scale Genomics Projects

A number of projects have attempted to follow up on the human genome project to varying degrees of success. The genomes have since been sequenced for a variety of model organisms, organisms of importance in health, agriculture, metagenomics of microorganisms (microbiome), ecology and conservation. The International HapMap Project, 1000 Genomes Project, and the 100K Genome Project aim to gather genetic variation data across human populations, along with gathering clinical and environmental variables for health and disease association studies. Whereas the ENCODE, ModENCODE, and FANTOM projects aim to characterise the functional aspects of human and model organism genomics. ENCODE in particular has attracted much criticism for over-inflated claims of DNA functionality. A notable finding of the ENCODE projects is that a high number of DNA sites bind to proteins or are transcribed into RNAs. However, these are not necessarily of functional importance. Conversely, the FANTOM projects approach this problem by focusing on expressed mRNAs, microRNAs, and epigenetic marks in each tissue or cell type. An area of recent interest are the long non-coding RNAs, the focus of FANTOM6, the next phase of the project

amenable to the CAGE-Seq technologies developed in prior FANTOM projects.

Other genomics databases have focused on facilitating distribution of genomic data generated by researchers, rather than generating it themselves. Genbank (NCBI) in the US, EMBL in Europe, and the DDBJ (NIG) in Japan do so by serving as repositories of DNA sequence data. GEO, arrayExpress, and caArray serve a similar purpose as a resource for gene expression datasets. These serve as a resource to support ongoing research to utilise data for genes of interest to particular research groups and further to make inferences based on larger datasets than accessible to any individual laboratory. These resources cover not only DNA sequence across the genome but also molecular profiles of other factors by adapting genomic sequencing or other high throughput technologies for quantifying gene expression or DNA methylation.

Similarly, international projects and consortiums have begun to release data gathered using common agreed upon protocols in laboratories across the world, often hosting public databases of these themselves, publishing their own investigations into the datasets as they are released, or offering basic searches and analytics of the data via a web portal. These databases include many of the genomics projects discussed above and the cancer-specific projects discussed below. In many ways, the quality, consistency, and accessibility of these international projects has become more appealing than accessing smaller studies, particularly for gene expression datasets where the more recent, larger projects have switched from microarray to RNA-Seq technologies. This distinction will also be discussed later.

1.1.5 Cancer Genomes

It's importance in the future of cancer research was noticed, even in the early days of genomics (Dickson, 1999). The Cancer Genome Project (CGP) based at Wellcome Trust Sanger Institute in the UK were among the first to launch investigations into cancer after the publication of the Human Genome, using this genome sequence, consensus across the cancer research literature, and sequencing the genes of cancers themselves. Initially, the Sanger Institute set out to sequence 20 genes across 378 samples while the Human Genome project was still ongoing (Collins and Barker, 2007), optimising sequencing and computation infrastructure for a larger project while doing so. The main aim of the Cancer Genome Project was to discover “cancer genes”, those frequently mutated in cancers by comparing the genes of cancer and normal tissue samples, both “oncogenes” and “tumour suppressors” which are activated and inactivated respectively in cancers. This project is ongoing and the UK continues to be involved in

international sequencing initiatives and those focused on particular tissue types.

The Sanger Institute also hosts the Catalogue of Somatic Mutations in Cancer (COSMIC), a database and website of cancer genes. This launch with 66,634 samples and 10,647 mutations from initial investigations into *BRAF*, *HRAS*, *KRAS2*, and *NRAS* (Bamford *et al.*, 2004). It has since expanded to include 1,257,487 samples with 4,175,878 gene mutations curated from 23,870 publications, including 29,112 whole genomes (Release v79 (23/08/2016 <http://cancer.sanger.ac.uk/cosmic>). This database now also identifies cancer genes from DNA copy number, differential gene expression and differential DNA methylation.

1.1.5.1 The Cancer Genome Atlas Project

Based in the US, the Cancer Genome Atlas (TCGA) project was established in 2005, a combined effort of the National Cancer Institute (NCI) and the National Human Genome Research Institute (NHGRI) of the National Institutes of Health (NIH). They first set out to demonstrate the pilot project on brain (2008), ovarian (2011), and lung (2012) cancers. In 2009, the project expanded aiming to analyse 500 samples each for 20-25 tumour tissue types. They have since exceeded that goal, with data available for 33 cancer types including 10 “rare” cancers, a total of over 10,000 samples.

The TCGA projects set out to generate a molecular “profile” of the tumour (and some matched normal tissue) samples: the genotype, somatic mutations, gene expression, DNA copy number, and RNA methylation levels. While these were originally performed largely with microarray technologies, exome and RNA-Seq has been since adopted and performed for many TCGA samples, with whole genomes being performed for some samples. Data which cannot be used to identify the patients (such as somatic mutation, expression, methylation, and various clinical factors) are publicly available.

1.1.5.2 The International Cancer Genome Consortium

TCGA and the Cancer Genome project in the UK are part of a larger International Cancer Genome Consortium (ICGC), now a concerted effort across 16 countries to sequence the genome, transcriptome, and epigenome of 50 tumour types from over 25,000 samples total. With some redundancy the following countries are profiling various tumour types: USA (including TCGA), China (16), France (10), Australia (4), South Korea (4), the UK (4), Germany (4), Canada (3), Japan (3), Mexico (3 in collaboration with the US), Singapore (2), Brazil, India, Italy, Saudi Arabia, and Spain. This is inherently international and several projects are collaborations, such as

between the USA and Mexico, Australia and Canada, Singapore and Japan, along with the UK and France representing the European Union. In order to avoid competing the existing TCGA projects, some countries focus on a particular cancer they have health interest: Australia (melanoma), Brazil (melanoma), India (oral), Saudi Arabia (thyroid), and Spain (CML). Others focus on a particular tissue subtype with poor prognosis: The UK (triple negative or Her2+ breast cancer), France (clear cell kidney), Australia and Canada (ductal Pancreas). Another approach is to focus on rare or child cancers: Canada, Italy, France, Germany, Japan and Singapore, and the US (TARGET project). Particularly countries in Asia (China, Japan, Singapore, and South Korea) have emphasised the value of adding tumour data from non-Western countries or non-European populations in addition the data from Europe and the TCGA in the US. Data from 9 of these countries is already available on the ICGC website with the project ongoing.

1.1.5.2.1 Findings of the TCGA and ICGC Projects

Text

1.1.6 Genomic Cancer Medicine

There is much anticipation in cancer research for genomics technologies to have a clinical impact in cancer medicine: from diagnosis and prognosis to treatment developments and strategies. These may result either from direct use of genome or RNA-Seq in clinical laboratories or indirectly from biomarkers and treatments developed with research facilitated by genomics. This second strategy is likely to have a more immediate patient benefit due to the cost of genome sequencing, particularly considering adoption in public healthcare systems with a limited budget.

1.1.6.1 Cancer Genes and Driver Mutations

There are two main categories of “cancer genes”. Oncogenes are those activated in cancers either by gain of function mutations in proto-oncogenes, amplification of DNA copies, or elevated gene expression. Their normal functions are typically to regulate stem cells or to promote cellular growth and recurrent mutations are typically concentrated to particular gene regions. Conversely, tumour suppressor genes are those inactivated in cancer either by loss of function mutations, deletion of DNA copies, repression of gene expression, or hypermethylation. Their normal functions are typically

to regulate cell division, DNA repair, and cell signalling.

Detecting these cancer genes is a major challenge in cancer biology and has been revolutionised by genomic technologies. Recurrent mutations, or DNA copy number variants and differential gene expression or DNA methylation are all indicative of cancer genes which can be detected in genomics data. Important “driver” cancer genes are difficult to detect from “passenger” mutations due to patient variation, tumour heterogeneity, and genomic instability. However, many cancer genes have been replicated from previous studies or well supported from genomics data. There remains the challenge of translating the identification of cancer genes to patient benefit with characterisation of variants of unknown significance, which mutation or gene expression markers can be used to monitor tumour progression or treatment response, and design of therapeutic intervention against many molecular targets for which they have yet to be developed or repurposed from other diseases to cancers.

1.1.6.1.1 Characterisation from Genomics Projects

e.g., recurrent mutations in TCGA / COSMIC, co-occurrence / mutual exclusivity, Pan Cancer / tissue-specificity

1.1.6.2 Personalised or Precision Cancer Medicine

The notion of using a patient’s genome to tailor healthcare to an individual has been appealing since the advent of genomics, popularised with the term “personalised medicine”. This approach was expected to span from preventative lifestyle advice to effective treatments. Personalised medicine was intended to contrast with current strategies of health advice, screening, prognostics, and treatments based on what works well with the majority of the population, highlighting that adverse effects of treatments occur in a significant subpopulation and that many clinical studies are dominated by Western populations of European ancestry and may not generalise to other populations.

While the importance of genomics is still recognised in translational cancer research, its potential has been emphasised particularly in molecular diagnosis, prognosis, and treatments of patients already presenting with cancers in the clinic rather than preventative medicine. This is in part due to the vast number of variants of unknown clinical significance, the ethical issue of reporting on incidental findings, and the regulatory issues direct-to-consumer genetics companies have encountered offering health risk assessment.

More recently the term “Genomic medicine” has been preferred to describe the paradigm of treating cancers by their genomic features, particularly grouping patients by the mutation, expression, or DNA methylation profiles of their cancers. Radical proponents advocate for these molecular subtypes to supersede tissue or cell type specific diagnosis of cancers. However, in practice they are often used in combination, with clinical and pathological factors being informative of prognosis and surgical training specialising by organ system. The related term of “precision medicine” also stems from this trend with the rationale to target these molecular subtypes with separate treatment strategies, particularly in developing and applying treatments targeted against a particular mutation specific to cancers. To this end much research in this field is focused on identifying mutations and gene expression signatures amenable to distinguishing cancers, particularly oncogenic driver mutations, and developing treatments against them.

1.1.6.2.1 Molecular Diagnostics and Pan-Cancer Medicine

There is growing support for the use of molecular tools such as mutations or gene expression signatures to diagnose tumour subtypes in replacement or addition to tissue of origin or histology. This is particularly important in breast cancer where analysis of molecular data detected several distinct “intrinsic subtypes” with differences in malignancy and patient outcome which were distinguished by molecular mechanisms rather than tissue or cellular phenotype (Parker/Perou). Conversely, common molecular mechanisms may be shared between cancers across tissue types as discovered by the “Pan cancer” studies, such as those conducted by the TCGA and ICGC projects, which combined molecular profiles across tissue types. The molecular subtypes could feasibly be included in clinic testing as a panel of biomarkers for diagnostics and prognosis. Such biomarkers also have the potential to monitor drug response or risk of recurrence. This also raises the need for development of treatments for targeting these molecular subtypes.

1.1.6.3 Targeted Therapeutics and Pharmacogenomics

Targeted therapies with specificity against a molecular target are emerging as precision cancer medicine. Molecular targets can be tested in laboratory conditions with RNA interference or pharmacological agents. Identification of molecular targets is important for developing novel anti-cancer treatments along with validation and drug testing. For oncogenic mutations, the recurrent mutant variant or overexpressed gene is directly in-

hibited using structure-aided drug design or compound screening. However, oncogenes with high homology to other genes or tumour suppressor genes (where lost in cancers) are not amenable to direct targeting (Kaelin, 2009).

Despite controversy over their prohibitively high cost (PHARMAC, 2016), targeted therapeutics have been applied as monoclonal antibodies against oncogenes (such as *HER2*) with relative success in clinical trials (Miles, 2001), generating considerable interest in wider application of this approach. Targeted therapeutics have potential to have applications across cancer tissue types, specificity against tumour cells, wide therapeutic windows, and combination therapies (even in advanced disease or as a chemopreventative in high-risk individuals).

1.1.6.3.1 Targeting Oncogenic Driver Mutations

Oncogene targeted therapies have also been developed with some examples of effective clinical application against cancers. However, they already begun to manifest problems with resistance, recurrence, tissue specificity, and design of inhibitors specific to oncogenic variants rather than proto-oncogene precursors. Targeted anticancer therapeutics can exploit complex interactions to distinguish normal and cancerous cells which may benefit from studies of gene regulation or interaction networks. The unexpected synergy between inhibitors of the oncogenes *BRAF*^{V600E} and *EGFR* in colorectal cancer is an example of such a system Prahallad *et al.* (2012).

Despite successful application of vemurafenib against *BRAF*^{V600E} in melanomas Dienstmann and Tabernero (2011); Ravnan and Matalka (2012), colorectal cancers with *BRAF*^{V600E} mutations have poor prognosis and lack drug response. Prahallad *et al.* (2012) used an RNAi screen and found that *EGFR* inhibition is synergistic with vemurafenib against *BRAF*^{V600E} in colon cell lines and xenografts due feedback activation of *EGFR*. Vemurafenib which induced rapid reactivation of MAPK/ERK signalling via *EGFR* in colorectal cell lines in a tissue-specific manner Corcoran *et al.* (2012), although these may be relevant to acquired resistance in melanoma Sun *et al.* (2014). Thus combination therapies against several molecular pathways may be necessary to anticipate acquired resistance Ravnan and Matalka (2012) and targeted therapeutics may be further refined from understanding the pathway structure and functional interactions cancer cells.

1.1.6.4 Systems and Network Biology

- systems biology

- complex networks
- shortest paths
- scale-free
- small world
- biological and molecular networks

The properties of large networks were studied by constructing random networks by randomly linking a fixed number of nodes (Erdős and Rényi, 1959, 1960). Despite the random nature of these networks, properties such as their connectivity were well characterised. The vertex degree of random network follows a Poisson distribution, however this property does not hold in nature, suggesting that natural networks are non-random or not formed in this way Barabási and Oltvai (2004).

This work formed the foundation for studying complex networks which model features of real world networks not found in Erdős and Rényi's random networks. The small world property, made popular by findings in social networks (Milgram 1967; Travers & Milgram 1969), is the remarkably short path lengths between any nodes in a small world network. A small world network is well-connected with a characteristic path length proportional to the logarithm of the number of nodes Watts and Strogatz (1998). (Watts and Strogatz, 1998) developed a model of random rewiring of a regular network to construct random networks with the small world property and a high clustering coefficient. While these properties are more representative of networks occurring in nature, their model is limited by the degree distribution which converges to a Poisson distribution as it is rewired Barrat and Weigt (2000).

The degree distribution of naturally occurring networks often follows a power law distribution with the majority of nodes having far fewer connections than average and a small subset of highly connected network 'hubs' Barabási and Albert (1999). Hubs further differentiate into 'party' hubs (which interact simultaneously with many partners) and 'date' hubs (which interact with different partners in different conditions) Han *et al.* (2004). Network hubs can also be classed as associative or dissociative depending on whether they tend toward or away from connecting directly to other network hubs (van Steen, 2010). The associative and dissociative properties can also be used to test whether nodes of a particular subgroup (e.g., gene function) associate with each other.

Barabási and Albert (1999) constructed a network model in an entirely different way to randomly generate scale-free networks which have a power law degree distribution. They constructed random networks by preferential attachment, modelling growth of a network by sequentially adding nodes with links to existing nodes. The scale-free nature of the random networks was ensured by adding new nodes with an increasing probability of attachment to an existing node if it has higher degree. These networks successfully capture the scale-free nature of many real world networks with short characteristic path length and low eccentricity resulting in super small worlds Barabási and Albert (1999). Scale-free networks are limited by a low clustering coefficient and lack of modular structure; however, they have enabled the study of scale-free network topology and served as a basis for modified scale-free models (Dorogovtsev and Mendes, 2003; Holme and Kim, 2002).

Han *et al.* (2004) observed dynamic modularity in biological networks and suggested the network structure may underpin genetic robustness and plasticity. They focus on network hubs which are more likely to be essential genes and define the subgroups of hubs based on correlation of gene expression with protein-protein interaction partners: ‘party’ hubs (which interact simultaneously with many partners) and ‘date’ hubs (which interact with different partners in different conditions). Party and date hubs occurred most frequently within and between network modules respectively. Party hubs were considered local regulators, whereas date hubs were considered important to network connectivity as global regulators. This distinction between classes of network hubs was supported by differences in tissue specificity and clinical relevance as a proposed predictor of clinical outcome in breast cancer with an AUROC of 0.784 Taylor *et al.* (2009). However, correlation between expression and protein interactions were not robustly reproduced. The importance of date hubs has been criticised for assuming a bimodal distribution and basing the global importance of data hubs on a small subset Agarwal *et al.* (2010). As an alternative interpretation, (Agarwal *et al.*, 2010) suggest the importance of interactions rather than network hubs as interactions important to the network were between functionally similar proteins. Network hubs can also be classed as associative or dissociative depending on whether they tend toward or away from connecting directly to other network hubs (van Steen 2010). The associative and dissociative properties can also be used to test whether nodes of a particular subgroup (e.g., gene function) associate with each other.

Applications of network theory are diverse, including uses in social sciences, engineering, and computer science. Due to their complexity and difficulty of gathering suffi-

cient empirical data, biological applications of network theory are relatively unexplored. High-throughput technologies such as siRNA screens, two-hybrid screens, microarrays and massively parallel sequencing have made generating genome-scale molecular data feasible and enabled analysis of biological networks at the molecular level. Many types of inter-related molecular networks can be constructed and analysed, depending on the biological application. Genetic interaction networks will be the focus of this project because they are relatively unexplored compared to other molecular networks, have potential for applications in drug discovery (particularly cancer treatment), and may lead to better understanding of the role of genetics in cellular function and disease. Genetic interactions are usually studied at a high-throughput scale in simple model organisms such as bacteria, yeasts or the nematode worm; studies in humans, mammals, and non-model organisms (where applications would have the most societal impact) are limited by cost, time and labour constraints. Computational approaches with effective predictive models are the only feasible approach to study the connectivity of a biological network in a complex metazoan cell at the genome-scale.

1.1.6.4.1 Network Medicine, and Polypharmacology

Molecular networks are biological networks consisting on biological molecules including genes, transcripts (with non-coding and microRNAs), or proteins related by known interactions and gene regulatory or metabolic pathways. Targeted therapeutics have had some success for drug discovery, particularly in anticancer applications, including exploiting these molecular networks by designing combination therapies and applying a network pharmacology framework Hopkins (2008). Rational design of drugs selective to a single target has often failed to deliver clinical efficacy. Many existing effective drugs modulate multiple proteins, having been selected for biological effects or clinical outcome rather than molecular targets. Proponents of network biology and polypharmacology (specific binding to multiple targets) recommend to develop drugs with a desired target profile designed for the target topology Hopkins (2008); Barabási and Oltvai (2004). Multi-target treatments aim to achieve a clinical outcome through modulation of molecular networks since the genetic robustness of a cell often compensates for loss of a single molecular target.

While multi-target drugs may be more difficult to design, they are faster to test clinically than drug combinations which are usually required to be tested separately first Hopkins (2008). Synthetic lethal treatments for cancer, drug combinations and multi-target drugs to combat resistance to chemotherapy and antibiotics can be in-

formed by biological networks Hopkins (2008); Barabási and Oltvai (2004). Further optimisation of timing and dosing of drug combinations may increase efficacy and needs to be explored for combination effects with low efficacy as separate treatments. Low doses and drug holidays are other counter intuitive approaches which may increase clinical efficacy, reduce adverse effects, and reduce drug resistance (Sun *et al.*, 2014; Tsai *et al.*, 2012).

A molecular map of the interactions and pathways in the mammalian cellular network has the potential to impact upon drug design and clinical practice, particularly in treatment of cancer and infectious disease. Characterisation of the target system and impact of existing treatments, such as *BRAF*^{V600E} and *EGFR* inhibitors, enable wider application of the mechanisms for such interventions exploiting genetic interactions or pathways. This could lead to development of more effective treatment interventions for these systems and prediction of similar molecular systems for development of novel drug targets and combinations.

1.2 A Synthetic Lethal Approach to Cancer Medicine

Synthetic lethality has vast potential to improve cancer medicine by expanding application of targeted therapeutics to include inactivation of tumour suppressors and genes that are difficult to target directly. Synthetic lethal interactions are also studied for gene function and drug mode-of-action in model organisms. This section introduces the concept of synthetic lethality as it was originally conceived and how it has been adopted conceptually in cancer research. Detecting these interactions at scale and interpreting them is the focus of this thesis, hence we start with an overview of the concepts involved, initial work on the interaction, and the rationale for applications to cancer. Specific investigations into synthetic lethality in cancer, detection by experimental screening, and prediction by computational analysis will then be reviewed.

1.2.1 Synthetic Lethal Genetic Interactions

Genetic interactions are a core concept of molecular biology, discovered among earliest investigations of Mendelian genetics, and receiving revived interest with new technologies and potential applications. Biological epistasis is the effect of an allele at one locus “masking” the phenotype of another locus (Bateson and Mendel, 1909). Statistical epistasis is where there is significant disparity between the observed and expected phenotype of a double mutant, compared to the respective phenotypes of single mu-

tants and the wild-type (Fisher, 1919). Fisher’s definition lends itself to quantitative traits and more broadly encompasses synthetic genetic interactions (SGIs). These have become popular for studies in yeast genetics and cancer drug design (Boone *et al.*, 2007; Kaelin, 2005).

Synthetic genetic interactions are substantial deviations of growth or viability from the expected null mutant phenotype (of an organism or cell) assuming additive (deleterious) effects of the single mutants. The double mutant does not necessarily have either single mutant phenotype (as shown for cellular growth phenotypes in Figure 1.1). Most SGIs are more viable than either single mutant or less viable than the expected double mutant. Mutations are “synergistic” in negative SGIs with more deviation from the wild-type than expected. Formally, “synthetic sick” (SSL) and “synthetic lethal” (SL) interactions are negative SGIs giving growth inhibition and inviability respectively. Synthetic lethality in cancer research more broadly describes any negative SGI with specific inhibition of a mutant cell, including SSL interactions. Mutations are “alleviating” in positive SGIs with less deviation from the wild-type than expected. For viability, “suppression” and “rescue” are positive SGIs giving at least partial restoration of wild-type growth from single mutants with growth impairment and lethal phenotypes respectively. Negative SGIs were markedly more common than positive SGIs in a number of studies in model systems Tong *et al.* (2004); Boucher and Jenna (2013).

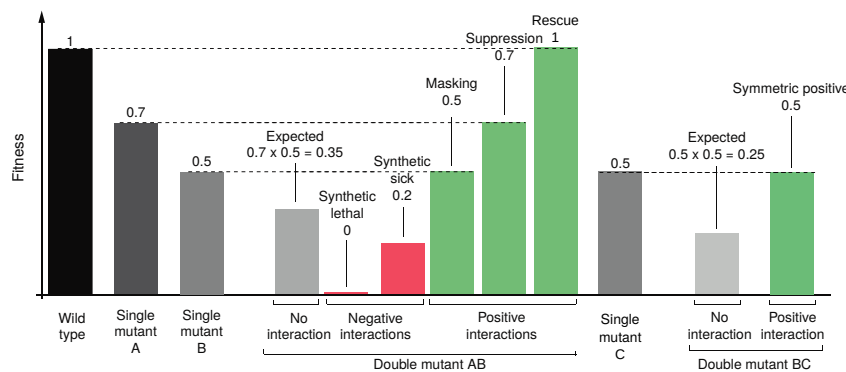


Figure 1.1: **Synthetic genetic interactions.** Impact of various negative and positive SGIs: negative interactions involve deleterious (sick) or inviable (lethal) phenotypes whereas positive interactions involve restoring viability by masking or suppressing the other mutation or complete rescue of the wildtype phenotype. Figure adapted from (Costanzo *et al.*, 2011) concerning growth viability fitness in yeast.

1.2.2 Synthetic Lethal Concepts in Genetics

Synthetic lethal genes are generally regarded to arise due to functional redundancy. Due to the functional level of SGIs, synthetic lethal genes do not need directly interact, nor be expressed in the same cell or at the same developmental stage: serving related functions is sufficient to affect cell (or organism) viability and be relevant to drug-mode-of-action cancer biology. Combined loss of genes performing an essential or important function in a cell are therefore deleterious. Synthetic lethal gene pairs are therefore pairwise essential with “induced essentiality”: each synthetic lethal gene becomes essential to the cell upon loss of the other.

Since synthetic lethal gene partners can be affected by extracellular stimuli and chemical, essentiality of synthetic lethal genes can be induced by the environment of a cell. An environmental stress conditions may inhibit one or the other synthetic lethal gene, such as exposure to chemicals, in which case the synthetic lethal partner gene is “conditionally essential” (Hillenmeyer, 2008). Thus the evolutionary rationale for the abundance of SGIs (compared to the surprisingly low number of essential genes) in a Eukaryotic genome attributed to genetic functional redundancy and network robustness of a cell which are advantageous to survival.

Biological functions are typically performed by a pathway of genes (or their products), may genes of the same pathway may be interchangeable as synthetic lethal partners of a particular gene since loss of the pathway is deleterious without the synthetic lethal partner gene. Therefore biological pathways can be subject to induced essentiality under loss of a gene and synthetic lethality be defined occur at pathway level or occur in a gene regulation network.

1.2.3 Studies of Synthetic Lethality

Genetic high-throughput screens have identified unexpected, functionally informative, and clinically relevant synthetic lethal interactions; including synthetic lethal partners of genes recurrently mutated in cancer or attributed to familial early-onset cancers. While screening presents an appealing strategy for synthetic lethal discovery, computational approaches are becoming popular as an alternative or complement to experimental methods to overcome inherent bias and limitations of experimental screens. An array of recently developed computational methods (Wang and Simon, 2013; Tiong *et al.*, 2014; Jerby-Arnon *et al.*, 2014; Lu *et al.*, 2015; Wappett, 2014) show the need for synthetic lethal discovery in the fundamental genetics and translational cancer research

community. However, existing computational methods are not suitable for queries of genomic data for interacting partners of a particular gene: they have been applied pairwise across the genome, do not have software released to apply the methodology, or lack statistical measures of error for further analysis. A robust prediction of gene interactions is an effective and practical approach at a scale of the entire genome for ideal translational applications, analysis of biological systems, and constructing functional gene networks.

1.2.3.1 Synthetic Lethal Pathways and Networks

SGIs are very common in genomes, with a 4-fold more interactions detected with synthetic gene array mating screens than protein-protein interactions yeast-2-hybrid studies (Tong *et al.*, 2004). The SGI network is scale-free with power-law vertex degree distribution and low average shortest path length (3.3) as expected for a complex biological network (Barabási and Oltvai, 2004). Highly connected “hub” genes with the highest number of links (vertex degree) are functionally important with many negative SGI hubs involved in cell cycle regulation and many positive SGI hubs involved in translation (Baryshnikova *et al.*, 2010; Costanzo *et al.*, 2010). Negative SGIs were far more common than positive SGIs, with synthetic gene loss being more likely to be deleterious to cell than advantageous which indicates that synthetic lethality may be comparably easier to detect than other SGIs.

Essential pathways are highly buffered with 5-fold more interactions than other SGIs, consistent with strong selection for survival, as found with conditional and partial mutations in essential genes (Davierwala *et al.*, 2005). This SGI network had scale-free topology and rarely shared interactions with the protein-protein interaction network. These networks are related by an “orthogonal” relationship: shared partners in one network tend to be themselves connected directly in the other network. Essential genes were likely to have closely related functions, whereas non-essential networks more relatively more inclined to have SGIs between distinct biological pathways.

1.2.3.1.1 Conservation and Evolution of Synthetic Lethality

There is poor conservation of specific SGIs between *S. cerevisiae* and *S. pombe* with 29% of the interactions tested in both distantly related species being conserved between them (Dixon2008). The remaining interactions show high species-specific differences; however, many of the species specific interactions were still conserved between biological pathways, protein complexes, or protein-protein interaction modules. Similarly,

conservation of pathway redundancy was also found between Eukaryotes (*S. cerevisiae*) and prokaryotes (*E. coli*) (Butland *et al.*, 2008). Negative SGIs were more likely to be conserved between biological pathways, whereas positive SGIs were more likely to be conserved within a pathway or protein complex (Roguev *et al.*, 2008).

A modest 5% of interactions were conserved between unicellular (*S. cerevisiae*) and multicellular (*C. elegans*) organisms but the nematode SGI network had similar scale-free topology and modularity despite difficulties metazoan RNAi screens being incomplete knockouts compared to null mutations in yeast (Bussey *et al.*, 2006). The nematode SGI screen identified network hubs with important interactions to orthologues of known human disease genes (Lehner *et al.*, 2006). Despite the lack of direct conservation of SGIs between yeasts and nematode worms, genetic redundancy at the gene or pathway level may yet be consistent with an induced essentiality model of SGIs where gene functions are conserved with network restructuring over evolutionary change (Tischler *et al.*, 2008). While nematode models are more closely related to human cells, cancer cells can present growth and viability phenotypes more comparable to yeast models. Therefore findings from both SGA and RNAi models are relevant to understanding cellular network structure and in healthy and cancerous human cells. RNAi has also been applied to human and mouse cancer cells in cell culture and genetic screening experiments. These findings suggest that SGI network “rewiring” is a concern for identifying specific synthetic lethal interactions in cancer and a pathway approach may be more robust in the context of evolution, patient variation, tumour heterogeneity, and disease progression.

1.2.4 Synthetic Lethal Concepts in Cancer

Loss of function occurs in many genes in cancers including tumour suppressors and yet few interventions target such mutations compared to targeted therapies for gain of function mutation in oncogenes (Kaelin, 2005). Synthetic lethality is a powerful design strategy for therapies selective against loss of gene function with potential for application against a range of genes and diseases (Kaelin, 2009; Fece de la Cruz *et al.*, 2015). Since synthetic lethality affects cellular viability by indirect functional relationships genes, it is suitable for indirectly targeting of mutations in cancers. Once synthetic lethal partners of cancer genes are identified, targeted therapeutics can be applied against them. When genes are disrupted in cancers, the induced essentiality of synthetic lethal partners is a vulnerability that may be exploited for anti-cancer therapy. This has the potential to be very specific against cancer cells (with the target

mutation) over non-cancer cells (with a functional compensating gene). Analogous to “oncogene addiction”, where cancer cells adapt to particular oncogenic growth signals and become reliant on them to remain viable (Luo *et al.*, 2009; Weinstein, 2000), synthetic lethal partners of inactivated tumour suppressors are required to maintain cancer cell viability and proliferation as such they are subject to “non-oncogene addiction” and are feasible anti-cancer drug targets.

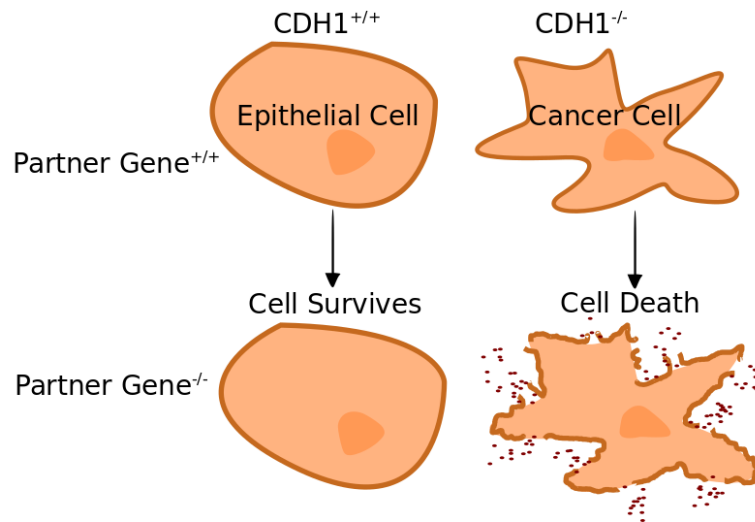


Figure 1.2: **Synthetic lethality in cancer.** Rationale of exploiting synthetic lethality for specificity against a tumour suppressor gene (e.g., *CDH1*) while other cells are spared under the inhibition of an SL partner gene.

The synthetic lethal approach to cancer medicine is most amenable to loss of function mutations in tumour suppressor genes, where it would feasibly be effective against any loss of function mutation across the tumour suppressor with a viable synthetic lethal partner gene (as shown in Figure 1.2). However, the approach may also be suitable for cases where cancer cells have mutations where the normal function of the gene is disrupted such as if it were overexpression (“synthetic dosage lethality”) or if an oncogene interfered with the function of the proto-oncogenic variant such as competitive inhibition. Thus synthetic lethality expands the range of cancer-specific mutations feasible to target with targeted therapeutics to absence of tumour suppressor genes and distinguishing highly homologous oncogenes by functional differences by targeting their synthetic lethal partners.

1.2.5 Clinical Impact of Synthetic Lethality in Cancer

The synthetic lethal interaction of *BRCA1* or *BRCA2* with *PARP1* in breast cancer is an example of how gene interactions are important in cancer, including translation to the clinic. These genetic interactions enable specific targeting of mutations in *BRCA1* or *BRCA2* tumour suppressor genes with PARP inhibitors by inducing synthetic lethality in breast cancer (Farmer *et al.*, 2005). PARP inhibitors are one of the first targeted therapeutics against a tumour suppressor mutation with success in clinical trials.

BRCA1 or *BRCA2* and *PARP1* genes demonstrate the application of the synthetic lethal approach to cancer therapy Ashworth (2008); Kaelin (2005). *BRCA1* and *BRCA2* are homologous DNA repair genes, widely known as tumour suppressors; mutation carriers have substantially increased risk of breast (risk by age 70 of 57% for *BRCA1* and 59% for *BRCA2*) and ovarian cancers (risk by age 70 of 40% for *BRCA1* and 18% for *BRCA2*) (Chen and Parmigiani, 2007). The *BRCA1* or *BRCA2* genes, which usually repair DNA or destroy the cell if it cannot be repaired, have inactivating somatic mutations in some familial and sporadic cancers. Poly-ADP-ribose polymerase (PARP) genes are tumour suppressor genes involved in base excision DNA repair. Loss of PARP activity results in single-stranded DNA breaks. However, *PARP1*^{-/-} knock-out mice are viable and healthy indicating low toxicity from PARP inhibition (Bryant *et al.*, 2005).

Bryant *et al.* (2005) showed that *BRCA2*^{-/-} cells were sensitive to PARP inhibition by siRNA of *PARP1* or drug inhibition (which targets *PARP1* and *PARP2*) using Chinese hamster ovary cells, MCF7 and MDA-MB-231 breast cell lines. This effect was sufficient to kill mouse tumour xenografts and showed high specificity to *BRCA2* deficient cells in culture and xenografts. Farmer *et al.* (2005) replicated these results in embryonic stem cells and showed that *BRCA1*^{-/-} cells were also sensitive to PARP inhibition relative to the wild-type with siRNA and drug experiments in cell culture and drug activity against *BRCA1* or *BRCA2* deficient embryonic stem cell mouse xenografts. They found evidence that PARP inhibition causes DNA lesions, usually repaired in wild-type cells, which lead to chromosomal instability, cell cycle arrest, and induction of apoptosis in *BRCA1* or *BRCA2* deficient cells. Therefore, the pathways cooperate to repair DNA giving a plausible mechanism for combined loss as an effective anti-cancer treatment.

Thus PARP inhibitors have potential for clinical use against *BRCA1* or *BRCA2* mutations in hereditary and sporadic cancers (Ashworth 2008; Kaelin2005). PARP inhibi-

tion has been found to be effective in cancer patients carrying *BRCA1* or *BRCA2* mutations and some other ovarian cancers, suggesting synthetic lethality between PARP and other DNA repair pathways (Ström and Helleday, 2012). This supports the potential for PARP inhibition as a chemo-preventative alternative to prophylactic surgery for high risk individuals with *BRCA1* or *BRCA2* mutations (Ström and Helleday, 2012). Hormone-based therapy has also been suggested as a chemo-preventative in such high risk individuals and aromatase inhibitors have completed phase I clinical trials for this purpose (Bozovic-Spasojevic2012). Ström and Helleday (2012) also postulate increased efficacy of PARP inhibitors in the hypoxic DNA-damaging tumour micro-environment.

A PARP inhibitor, olaparib, showed fewer adverse effects than cytotoxic chemotherapy and anti-tumour activity in phase I trials against *BRCA1* or *BRCA2* deficient familial breast, ovarian, and prostate cancers (Fong *et al.*, 2009) and sporadic ovarian cancer (Fong *et al.*, 2010). AstraZeneca has reported phase II trials showing the treatment is effective in *BRCA1* or *BRCA2* deficient breast (Tutt *et al.*, 2010) and ovarian cancers (Audeh *et al.*, 2010) with a favourable therapeutic window and similar toxicity between carriers of *BRCA1* or *BRCA2* mutations and sporadic cases. AstraZeneca announced that olaparib has begun phase III trials for breast and ovarian cancers in 2013. Mixed results in phase II trials in ovarian cancer are behind the delays addressed by retrospective analysis of the cohort subgroup with confirmed mutation of *BRCA1* or *BRCA2* genes in the tumour; unsurprisingly these patients, benefit most from the PARP inhibitor treatment and have increased platinum sensitivity in combination treatment. This demonstrates the clinical impact of a well characterised system of synthetic lethality with known cancer risk genes. Synthetic lethality has the benefit of being effective against inactivation of tumour suppressor genes by any means, broader than targeting a particular oncogenic mutation (Kaelin, 2005). The targeted therapy is effective in both sporadic and hereditary *BRCA1* or *BRCA2* deficient tumours acting against an oncogenic molecular aberration across several tissues.

[Update re. FDA approval for Ovarian]

These PARP inhibitors are FDA approved for some cancers McLachlan *et al.* (2016), are effective against germline and sporadic *BRCA1* or *BRCA2* mutations, and are a potential prevention alternative to prophylactic surgery for high risk mutation carriers Ström and Helleday (2012).

1.2.6 High-throughput Screening for Synthetic Lethality

The function of signalling pathways and combinations of interacting genes are important in cancer research but classical genetics approaches have been limited to non-redundant pathways (Fraser, 2004). The emerging RNAi technologies have vastly expanded the potential for studying genetic redundancy in mammalian experimental models including testing experimentally for synthetic lethality (Fraser, 2004). Identifying synthetic lethality is crucial to study gene function, drug mechanisms, and design novel therapies (Lum *et al.*, 2004). Candidate selection of synthetic lethal gene pairs relevant to cancer has shown some success but is limited because interactions are difficult to predict; they can occur between seemingly unrelated pathways in model organisms (Costanzo *et al.*, 2011). While biologically informed hypotheses have had some success in synthetic lethal discovery (Bitler *et al.*, 2015; Bryant *et al.*, 2005; Farmer *et al.*, 2005), interactions occurring indirectly between distinct pathways would be missed (Boone *et al.*, 2007; Costanzo *et al.*, 2011). Scanning the entire genome for interactions against a clinically relevant gene is an emerging strategy being explored with high-throughput screens (Fece de la Cruz *et al.*, 2015) and computational approaches (Boucher and Jenna, 2013; van Steen, 2012).

Experimental screening for synthetic lethality is an appealing strategy for wider discovery of functional interactions *in vivo* despite many potential sources of error which must be considered. The synthetic lethal concept has both genetic and pharmacological screening applications to cancer research. Genetic screens, with RNAi to discover the specific genes involved, inform development of targeted therapies with a known mode of action, anticipated mechanisms of resistance, and biomarkers for treatment response. RNAi is a transient knockdown of gene expression more similar to the effect of drugs than complete gene loss and makes comparison to screens in model organisms difficult (Bussey *et al.*, 2006). The RNAi gene knockdown process has inherent toxicity to some cells, potential off-target effects, and issues with a high false positive rate. Therefore, it is important to validate any candidates in a secondary screen and replicate knockdown experiments with a number of independent shRNAs. Alternative gene knockout procedures have also been proposed for synthetic lethal screening including a genome-wide application of the CRIPR/Cas9/sgRNA genome editing technology (Sander and Joung, 2014), episomal gene transfer (Vargas *et al.*, 2004), or RNAi with lentiviral transfection for delivery of shRNA (Telford *et al.*, 2015). Genetic screens have potential for quantitative gene disruption experiments to selectively target overexpressed genes in

cancer via synthetic dosage lethality. While powerful for understanding fundamental cellular function, analysis of isogenic cell lines is inherently limited by assuming only a single mutation differs between them despite susceptibility to “genetic drift” and cannot account for diverse genetic backgrounds or tumour heterogeneity (Fece de la Cruz *et al.*, 2015). Genetic screens thus identify targets to develop or repurpose targeted therapies for disease but alone will not directly identify a lead compound to develop for the market or clinical translation.

Chemical screens are immediately applicable to the clinic by directly screening for selective lead compounds with suitable pharmacological properties. However chemical screens lack a known mode of action, may affect many targets, and screen a narrow range of genes with existing drugs. With either approach there are many challenges translating candidates into the clinic such as finding targets relevant to a range of patients, validation of targets, accounting for a range of genetic (and epigenetic) contexts or tumour micro-environment, identifying effective synergistic combinations, enhancers of existing radiation or cytotoxic treatments, avoiding inherent or acquired drug resistance, and developing biomarkers for patients which will respond to synthetic lethal treatment, including integrating these into clinical trials and clinical practice. Identifying specific target genes is an effective way to anticipate such challenges, which can be approached with genetic screens, so we will focus on these and computational alternatives. Screening methods have proven a fruitful area of research, despite being costly, laborious, and having many different sources of error. These limitations suggest a need for complementary computational approaches to synthetic lethal discovery.

1.2.6.1 Examples of Synthetic Lethal Screens

Overexpression of genes is another suitable application for synthetic lethality since overexpressed genes cannot be distinguished from the wild-type by direct sequence specific targeted therapy. Overexpression of oncogenes, such as *EGFR*, *MYC*, and *PIM1*, has been found to drive many cancers. *PIM1* is a candidate for synthetic lethal drug design in lymphomas and prostate cancers, where it interacts with *MYC* to drive cancer growth. van der Meer *et al.* (2014) performed an RNAi screen to for synthetic lethality between *PIM1* overexpression and gene knockdown in RWPE prostate cancer cell lines. *PLK1* gene knockdown and drug inhibition was an effective as a specific inhibitor of *PIM1* overexpressing prostate cells in cell culture and mouse tumour xenografts. *PLK1* inhibition reduced *MYC* expression in pre-clinical models, consistent with expression in human tumours which *PIM1* and *PLK1* are co-expressed

and correlated with tumour grade. Thus RNAi screening was valuable to identify a therapeutic targets and biomarkers for patient response as demonstrated with the finding of *PLK1* as a candidate drug target against prostate cancer progression.

Hereditary leiomyomatosis and renal cell carcinoma (HLRCC) is a cancer syndrome of predisposition to benign tumours in the uterus and risk of malignant cancer of the kidney attributed to inherited mutations in fumarate hydratase (*FH*). Boettcher *et al.* (2014) performed an RNAi screen on HEK293T renal cells for synthetic lethality with *FH*. They found enrichment of haem metabolism (consistent with the literature) and adenylate cyclase pathways (consistent with cAMP dysregulation in *FH* mutant cells). Synthetic lethality between *FH* mutation and adenylate cyclases was validated with gene knockdown, drug experiments, and replicated across both HEK293T renal cells and VOK262 cells derived from a HLRCC patient, suggesting new potential treatments against the disease.

Similarly, hereditary diffuse gastric cancer (HDGC) is a cancer syndrome of predisposition to early-onset malignant stomach and breast cancers attributed to mutations in E-cadherin (*CDH1*). Telford *et al.* (2015) performed an RNAi screen on MCF10A breast cells for synthetic lethality with *CDH1*. They found enrichment of G-protein coupled receptors (GPCRs) and cytoskeletal gene functions. The results were consistent with a concurrent drug compound screen with a number of candidates validated by lentiviral shRNA gene knockdown and drug testing including inhibitors of Janus kinase, histone deacetylases, phosphoinositide 3-kinase, aurora kinase, and tyrosine kinases. Therefore the synthetic lethal strategy has potential for clinical impact against HDGC, with particular interest in interventions with low adverse effects for chemoprevention, including repurposing existing approved drugs for activity against *CDH1* deficient cancers.

RNAi screening for synthetic lethality is also useful for functional genetics to understand drug sensitivity. Aarts *et al.* (2015) screened WiDr colorectal cells for synthetic lethality between *WEE1* inhibitor treatment and an RNAi library of 1206 genes with functions known to be amenable to drug treatment or important in cancer such as kinases, phosphatases, tumour suppressors, and DNA repair (a pathway *WEE1* regulates). Screening identified a number of synthetic lethal candidates including genes involved in cell cycle regulation, DNA replication, repair, homologous recombination, and Fanconi anaemia. Synthetic lethality with cell-cycle and DNA repair genes was consistent with the literature and validation in a panel of breast and colorectal cell lines supported checkpoint kinases, Fanconi anaemia, and homologous recombination

as synthetic lethal partners of *WEE1*. These results show that synthetic lethality can be used to improve drug sensitivity as a combination treatment, especially to exploit genomic instability and DNA repair, which are known to be clinically applicable from previous results with *BRCA1* or *BRCA2* genes and PARP inhibitors (Lord *et al.*, 2015). Therefore, *WEE1* inhibitors are an example of treatment which could be repurposed with the synthetic lethal strategy and similar findings would be valuable to clinicians as a source of biomarkers and novel treatments. While using a panel of cell lines to replicate findings across genetic background is a promising approach to ensure wide clinical application of validated synthetic lethal partners, a computational approach may be more effective as it could account for wider patient variation than scaling up intensive experiments on a wide array of cell lines and could screen beyond limited candidates from an RNAi library.

Chemical genetic screens are also a viable strategy to identify therapeutically relevant synthetic lethal interactions. Bitler *et al.* (2015) investigated *ARID1A* mutations, aberrations in chromatin remodelling known to be common in ovarian cancers, for drug response. Ovarian RMG1 cells were screened for drug response specific to *ARID1A* knockdown cells. They used *ARID1A* gene knockdown for consistent genetic background, with control experiments and 3D cell culture to ensure relevance to drug activity in the tumour micro-environment. Screening a panel of commercially available drugs targeting epigenetic regulators found *ESH2* methyltransferase inhibitors effective and specific against *ARID1A* mutation with validation in a panel of ovarian cell lines. Synthetic lethality between *ARID1A* and *ESH2* was supported by decreases in H3K27Me3 epigenetic marks and markers of apoptosis in response to *ESH2* inhibitors. This was mechanistically supported with differential expression of *PIK3IP1* and association of both synthetic lethal genes with the *PIK3IP1* promoter identifying the PI3K-AKT signalling pathway as disrupted when both genes are inhibited. This successfully demonstrates the importance of synthetic lethality in epigenetic regulators, identifies a therapeutically relevant synthetic lethal interaction, and shows that chemical genetic screens could model drug response and combination therapy in cancer cells. However this approach is limited to finding synthetic lethal interactions between genes with known similar function, which may not be the most suitable for treatment. Further limiting experiments to genes with existing targeted drugs reduces the number of synthetic lethal interactions detected, assumes on their drug specificity to a particular target, and many of these drugs are not clinically available yet anyway as they are still in clinical trials for other diseases or are not supported by healthcare systems in many

countries.

The examples above show that high-throughput screens are an effective approach to discover synthetic lethality in cancer with a wide range of applications. Screens are more comprehensive than hypothesis-driven candidate gene approaches and successfully find known and novel synthetic lethal interactions with potential for rapid clinical application. They have the power to test mode of action of drugs, find unexpected synthetic lethal interactions between pathways, or identify effective treatment strategies without needing a clear mechanism. However, synthetic lethal screens are costly, labour-intensive, error-prone, and biased towards genes with effective RNAi knockdown libraries. Limited genetic background, lethality to wild-type cell during gene knock-down, off-target effects, and difficulty replicating synthetic lethality across different cell lines, tissues, laboratories, or conditions stems from a high false positive rate and a lack of standardised thresholds to identify synthetic lethality in a high-throughput screen. Therefore there is a need for replication, validation, and alternative approaches to identify synthetic lethal candidates. Varied conditions between experimental screens and differences between RNAi or drug screens makes meta-analysis difficult. Thus genome-scale synthetic lethal experiments are not feasible, even in model organisms, so a computational approach would be more suitable for this task.

1.2.7 Computational Prediction of Synthetic Lethality

Prediction of gene interaction networks is a feasible alternative to high-throughput screening with biological importance and clinical relevance. There are many existing methods to predict gene networks, as reviewed by van Steen (2012) and Boucher and Jenna (2013) and summarised in Table ?? below. However, many of these methods have limitations including the requirement for existing SGI data, several data inputs, and reliability of gene function annotation. Many of the existing methods also assume conservation of individual interactions between species, which has been found not to hold in yeast studies (Dixon *et al.*, 2008). Tissue specificity is important in gene regulation and gene expression, which are used as predictors of genetic interaction. However, tissue specificity of genetic interactions cannot be explored in yeast studies and has not been considered in any of the following studies of multicellular model organisms, human networks, or cancers. Similarly, investigation into tissue specificity of protein-protein interactions (PPIs), an important predictor of genetic interactions, is difficult given the high-throughput two-hybrid screens occur out of cellular context for multicellular organisms.

Table 1.2: Methods for predicting synthetic lethality in cancer

Method	Input Data	Source	Tool Offered
Network Centrality	protein-protein interactions	Kranthi <i>et al.</i> (2013)	
Differential Expression	Expression Mutation	Wang and Simon (2013)	
Comparative Genomics	Yeast synthetic gene interactions	Heiskanen and Aittokallio (2012)	
Chemical-Genomics	Homology		
Comparative Genomics	Yeast synthetic gene interactions Homology	Deshpande <i>et al.</i> (2013)	
Machine Learning		Discussed by Babyak (2004) and Lee and Marcotte (2009)	
Differential Expression	Expression	Tiong <i>et al.</i> (2014)	
Literature Database		Li <i>et al.</i> (2014)	Syn-Lethality
Meta-Analysis	Meta-Analysis Machine Learning	Wu <i>et al.</i> (2014)	MetaSL
Pathway Analysis		Zhang <i>et al.</i> (2015)	
Protein Domains	Homology	Kozlov <i>et al.</i> (2015)	
Data-Mining Machine Learning	Expression Somatic mutation and DNA CNV siRNA in cell lines	Jerby-Arnon <i>et al.</i> (2014) Ryan <i>et al.</i> (2014) Crunkhorn (2014) Lokody (2014)	DAISY (method)
Genome Evolution Hypothesis Test Machine Learning	Expression DNA CNV Known SL	Lu <i>et al.</i> (2013) Lu <i>et al.</i> (2015)	
Bimodality	Expression DNA CNV Somatic Mutation	Wappett (2014) Wappett <i>et al.</i> (2016)	BiSEp
Chi-Squared Test	Expression (microarray) Somatic mutation	Tom Kelly, Parry Guilford, and Mik Black Dissertation (Kelly 2013)	SLIPT

There are a number of existing computational methods for predicting synthetic lethal gene pairs. While these demonstrate the power and need for predictions of synthetic lethality in human and cancer contexts, limitations of previous methods could be met with a different approach. For instance, computational approaches to synthetic lethal prediction are often difficult to interpret, replicate for new genes, or reliant on data types not available for genes that other cancer researchers work on.

Kranthi *et al.* (2013) took a network approach to discovery of synthetic lethal candidate selection applying the concept to “centrality” to a human PPI network involving interacting partners of known cancer genes. The effect of removing pairs of genes on connectivity of the network was used as a surrogate for viability which is supported by observations that the PPI and synthetic lethal networks are orthogonal in *S. cerevisiae* studies (Tong *et al.*, 2004). While they showed the power law distribution expected of a scale-free synthetic lethal network with centrality measures, their approach was

limited to known cancer genes and is not applicable to genes without PPI data. Other nucleotide sequencing data types are more commonly available for cancer studies at a genomic scale. Of further concern is that the results were enriched for p53 synthetic lethal partners which is relevant to many cancer researchers but this genome-wide approach did not detect many other cancer genes due to multiple testing. This enrichment may be due to the known drastic effect of removing p53 itself from the network as a master regulator, cancer driving tumour suppressor gene, and highly connected network “hub”. The focus on cancer genes is useful for translation into therapeutics but does not account for variable genetic backgrounds or effect of protein removal on the whole cellular network.

A comparative genomics approach by Deshpande *et al.* (2013) used the results of well characterised high-throughput mutation screens in *S. cerevisiae* as candidates for synthetic lethality in humans (Baryshnikova *et al.*, 2010; Costanzo *et al.*, 2010, 2011; Tong *et al.*, 2001, 2004). Yeast synthetic lethal partners were compared to human orthologues to find cancer relevant synthetic lethal candidate pairs with direct therapeutic potential. Proposed as a complementary approach to siRNA screens, several synthetic lethal candidates were successfully validated in cell culture; however, this methodology is limited to application on human genes with known yeast orthologues. Synthetic lethal interactions themselves may not be conserved between species Dixon *et al.* (2009), although synthetic lethal interactions between pathways may be more comparable. There have been many gene duplications in the separate evolutionary histories of humans and yeast which may lead to differences in genetic redundancy. Yeast are further not an ideal human cancer model because they do not have tissue specificity, multicellular gene regulation, or orthologues to a number of known cancer genes such as p53.

Differential gene expression has also been explored to predict synthetic lethal pairs in cancer which would be widely applicable due to the availability of public gene expression data for a large number of samples and cancer types. Wang and Simon (2013) found differentially expressed genes between tumours with or without functional p53 mutations in Cancer Genome Atlas (TCGA) and Cell Line Encyclopaedia (CCLE) RNA-Seq gene expression data as candidate synthetic lethal partner pathways of p53. Some of these pathways were consistent with the literature and drug sensitivity cell-line screens demonstrating the potential of gene expression as a surrogate for gene function and use of public genomic data to predict synthetic lethal gene pairs in cancer. However, the analyses were limited to kinase genes and focused on currently druggable

genes, lacking wider application of synthetic lethal prediction methodology. This approach may not be feasible or applicable in cancer genes with a lower mutation rate than p53.

Tiong *et al.* (2014) also investigated gene expression as a predictor of synthetic lethal pairs with colorectal cancer microarrays. Simultaneously differentially expressed “tumour dependent” gene pairs between cancer and normal tissue were used as candidate synthetic lethal interactions. The top 20 genes were tested for differential expression at the protein level with immunohistochemistry staining and correlation with clinical characteristics. Some of the predicted synthetic lethal pairs were consistent with the literature and 2 novel synthetic lethal interactions with p53 were validated in pre-clinical models. While a valuable proof-of-concept for integration of *in silico* approaches to synthetic lethal discovery in cancer, the results again focus on p53 rather than the wider application of synthetic lethal prediction. The gene expression analyses were conducted in a Han Chinese population with a small sample size (70 tumour, 12 normal) and may not be applicable to other populations.

Another approach to systematic synthetic lethality discovery specific to human cancer (in contrast to the plethora of yeast synthetic lethality data) was to build a database as done by Li *et al.* (2014). In their relational database, called “Syn-lethality”, they have curated both known experimentally discovered synthetic lethal pairs in humans (113 pairs) from the literature and those predicted from synthetic lethality between orthologous genes in *S. cerevisiae* yeast (1114 pairs). This knowledge-based database is the first known dedicated to human cancer synthetic lethal interactions and integrates gene functional, annotation, pathway and molecular mechanism data with experimental and predicted synthetic lethal gene pairs. This combination of data sources is intended to tackle the trade-off between more conclusive synthetic lethal experiments in yeast and more clinically relevant synthetic lethal experiments in human cancer models, such as RNAi, especially when high-throughput screens are costly and prone to false positives in either system and difficult to replicate across gene backgrounds. This database centralises a wealth of knowledge scattered in the literature including cancer relevant genes (*BRCA1*, *BRCA2*, *PARP1*, *PTEN*, *VHL*, *MYC*, *EGFR*, *MSH2*, *KRAS*, and *TP53*) and is publicly available as a Java App. However, the methodology was not released, so it is not possible to replicate their results, nor to add to the findings with new datasets. Suggested future directions were promising, such as constructing networks of known synthetic lethality, applying known synthetic lethality to cancer treatment, data mining, replicating the approach for synthetic lethality in model or-

ganisms, signalling pathways, and develop a complete global network in human cancer or yeast (both of which are still incomplete with experimental data).

Table 1.3: Machine learning methods used by Wu *et al.* (2014)

Method	Source	Tool Offered
Random Forest	Breiman (2001)	
Random Forest	Hall <i>et al.</i> (2009)	WEKA
J48 (decision tree)		
Bayes (Log Regression)		
Bayes (Network)		
PART (Rule-based)		
RBF Network		
Bagging / Bootstrap		
Classification via Regression		
Support Vector Machine (Linear)	Vapnik (1995)	
Support Vector Machine (RBF – Gaussian)	Joachims (1999)	
Multi-Network Multi-Class (MNMC)	Pandey <i>et al.</i> (2010)	
MetaSL (Meta-Analysis)	Wu <i>et al.</i> (2014)	MetaSL

From the same group, Wu *et al.* (2014) developed a meta-analysis method (based on the machine learning methods in Table 1.3) for synthetic lethal gene pairs relevant to developing selective drugs against human cancer. They note that computational approaches scale-up across the genome at lower cost than experimental screens but existing methods are limited by noise and overfitting to a particular predictive feature. Their “metaSL” approach performs well with an AUROC of 0.871 with the claimed strengths of existing machine learning methods and the results are shared on the web. However, once again, the method is not available for analysis of other genes studied by the cancer research community and the method lacks mechanisms, reproducibility, and interpretation by researchers. While machine learning has great potential as a predictor, it is difficult to interpret which features are being used for prediction and their mechanistic significance, particularly for biologists with limited exposure to computational concepts.

Focusing on the potential for synthetic lethality to be an effective anti-cancer drug target, Zhang *et al.* (2015) used modelling signalling pathways to identify synthetic lethal interactions between known drug targets and cancer genes. A computational approach was again used here to tackle the limitations of experimental RNAi screens such as scale, instability of knockdown, and off-target effects. This ‘hybrid’ method of

a data-driven model and known signalling pathways showed potential as a means to predict cell death in single and combination gene knockouts. They used time series gene expression data (Lee *et al.*, 2012) and Gene Ontology (GO) pathways Ashburner *et al.* (2000); Blake *et al.* (2015). This approach successfully detected many known essential genes in the human gene essentiality database, known synthetic lethal partners in the Syn-Lethality database, and predicted novel synthetic lethal gene pairs. Novel results were enriched for *TP53* and AKT synthetic lethal partners, genes known to be important in many cancers but also predicted to be essential by single gene disruption having a large impact on the signalling pathways. Notably, they claim to be able to detect functionally related pathways and protein complexes across the molecular function, cellular component, and biological process annotations provided by GO. The results were consistent with the experimental results in the literature but the validation for novel synthetic lethal interactions have not been validated yet.

While the mathematical reasoning and algorithms are given, the code was not released to replicate the findings or apply the methodology beyond the signalling pathways discussed by Zhang *et al.* (2015). The authors note limitations as directions for further research including the potential of their method to detect mechanisms, types of interactions, impact of activation or inhibition of proteins, and improve performance with a Boolean network or differential equation approach, all of which have been claimed but not shown. Further, this approach is limited by existing pathway data with limited scale, scope, and reliability coming from a range of sources. So far, modelling has been restricted to signalling pathways which are immediately applicable to cancer; while important, the approach lacks broader application to other diseases and pathway types. Zhang *et al.* (2015) also lack validation, replication, or application of findings and are heavily reliant on existing literature for testing their predictions.

Recognising the utility of synthetic lethality to drug inhibition and specificity of anti-cancer treatments, Jerby-Arnon *et al.* (2014) also saw the need for effective prediction of gene essentiality and synthetic lethality to augment experimental studies of SL. They developed a data-driven pipeline called DAISY (data mining synthetic lethality identification pipeline) and tested for genome-wide analysis of synthetic lethality in public cancer genomics data from TCGA and CCLE. DAISY is intended to predict the candidate synthetic lethal partners of a query gene such as genes recurrently mutated in cancer.

Jerby-Arnon *et al.* (2014) combined a computational approach to triage candidates with a conventional RNAi screen to validate synthetic lethal partners. They screened

a selection of computationally predicted candidates and randomly selected genes with RNAi against *VHL* loss of function mutation in RCC4 renal cell lines. The computational method had a high AUROC of 0.779 and predictions were enriched 4x for validated RNAi hits over randomly selected genes. This approach detected known synthetic lethal pairs such as *BRCA1* or *BRCA2* genes with *PARP1* and *MSH2* with *DHFR*. The synthetic lethal candidates identified with both RNAi screening and computational prediction formed an extensive network of 2077 genes with 2816 synthetic lethal interactions and similar network of 3158 genes with 3635 synthetic dosage lethal interactions (for synthetic lethality with over-expression). Each network was scale-free as expected of a biological network and was enriched for known cancer genes, essential genes in mice, and could be harnessed for predicting prognosis and drug response. While demonstrating the feasibility of combining experimental and computational approaches to synthetic lethality in cancer, there remain challenges in predicting synthetic lethal genes, novel drug targets, and translation into the clinic.

The DAISY methodology (Jerby-Arnon *et al.*, 2014) compares the results of analysis of several data types to predict synthetic lethality, namely: DNA copy number and somatic mutation for TCGA patient samples and CCLE cell lines. The cell lines were also analysed with gene expression and gene essentiality (shRNA screening) profiles. Genes were classed as inactivated by copy number deletion, somatic loss of function mutation, or low expression and tested for synthetic lethal gene partners which are either essential in screens or not deleted with copy number variants. Co-expression is also used for synthetic lethality prediction based on studies in yeast (Costanzo *et al.*, 2010; Kelley and Ideker, 2005). Copy number, gene expression and, essentiality analyses are stringently compared by adjusting each for multiple tests with Bonferroni correction and only taking hits which occur in all analyses. This methodology was also adapted for synthetic dosage lethality by testing for partner genes where genes are overactive with high copy number or expression. As discussed above, the predictions performed well and an RNAi screen for the example of *VHL* in renal cancer validated predicted synthetic lethal partners of *VHL* demonstrating the feasibility of combining approaches to synthetic lethal discovery in cancer and using computational predictions to enable more efficient high-throughput screening. DAISY performs well statistically with a AUROC of 0.779 on a set of gene pairs with experimental screen data, although co-expression and shRNA functional examination contributes much less of this than the mutation and copy number analysis (AUROC 0.683 alone). However, this methodology is very stringent, missing potentially valuable synthetic lethal candidates, may not be

applicable to genes of interest to other groups and the software for the procedure is not publicly released for replication.

Although the DAISY procedure performs well and has been well received by the scientific community (Crunkhorn, 2014; Lokody, 2014; Ryan *et al.*, 2014), showing a need for such methodology, there is no indication of adoption of the methodology in the community yet. The co-expression analysis may not be the most effective way to test gene expression for directional synthetic lethal interactions (where inverse correlation would be expected). In the interests of a large sample size, tissue types were not tested separately despite tissue-specific synthetic lethality being likely since gene function (and by extension expression, isoforms, and clinical characteristics) in cancers may often be tissue-dependent. Some data forms and analyses used, such as gene essentiality, may not be available for all cancers, genes, or tissues, and may not be reproduced.

Lu *et al.* (2015) critique the assumption of co-expression in the DAISY methodology and propose an alternative computational prediction of synthetic lethality based on machine learning methods and a cancer genome evolution hypothesis. Using DNA copy number and gene expression data from TCGA patient samples, a cancer genome evolution model assumes that synthetic lethal gene pairs behave in 2 distinct ways in response to an inactive synthetic lethal partner gene, either a “compensation” pattern where the other synthetic lethal partner is overactive or a “co-loss underrepresentation” pattern where the other synthetic lethal partner is less likely to be lost, since loss of both genes would cause death of the cancer cell. During the cancer genome evolution as the cell becomes addicted to the remaining synthetic lethal partner due to induced gene essentiality. These patterns would explain why DAISY detects only a small number of synthetic lethal pairs, compared to the large number expected based on model organism studies (Boone *et al.*, 2007), and the disparity between screening and computationally predicted synthetic lethal candidates due to testing different classes of synthetic lethal gene pairs.

Lu *et al.* (2015) compared a genome-wide computational model of genome evolution and gene expression patterns to the experimental data of Vizeacoumar *et al.* (2013) and Laufer *et al.* (2013). This simpler model performing well with an AUROC of 0.751 but was less than DAISY, although it did not rely on data from cell lines which may not represent patient disease. They predict a larger comprehensive list of 591,000 human synthetic lethal partners with a probability score threshold of 0.81, giving a precision of 67% and 14x enrichment of synthetic lethal true positives compared to randomly selected gene pairs. Discovery of such a vast number of cancer-relevant synthetic lethal

interactions in humans would not be feasible experimentally and is a valuable resource for research and clinical applications. These predictions are not limited by assuming co-expression of synthetic lethal partners or evolutionary conservation with model organisms enabling wider synthetic lethal discovery. However, there remains a lack of basis for an expectation of how many synthetic lethal partners a particular gene will have, how many pairs there are in the human genome, and whether pathways or correlation structure would influence predicted synthetic lethal partners.

Large scale, computational approaches have yet to determine whether synthetic lethal interactions are tissue-specific since Lu *et al.* (2015) used pan-cancer data for 14136 patients with 31 cancer types. Experimental data used for comparison was a small training dataset specific to colorectal cancer, and based on screens for other phenotypes, which may limit performance of the model or application to other cancers. Proposed expansion of the computational approach to mutation, microRNA, or epigenetic modulation of gene function and tumour micro-environment or heterogeneity suggests that synthetic lethal discovery could be widely applied to the current challenges in cancer genomics. This approach was also based on machine learning methodology and not supported by a software released for the community to develop, contribute to, or reproduce beyond the gene pairs given in the supplementary results.

Wappett *et al.* (2016) ...

1.3 E-cadherin as a Synthetic Lethal Target

E-cadherin is a transmembrane protein (encoded by *CDH1*) with several characterised functions in the cytoskeleton and cell-to-cell signaling. Here we outline the key known functions of E-cadherin and its importance in cancer biology. *CDH1* is a tumour suppressor gene, with loss of function occurring in both familial (germline mutations) and sporadic (somatic mutations) cancers. As such, *CDH1* inactivation is a prime example of a genetic event that could be targeted by synthetic lethality for anti-cancer treatments. Most notably this includes patients at risk of developing hereditary breast and stomach cancers for which conventional surgical or cytotoxic chemotherapy is not ideal (due to impact of quality of life) and who have a known genetic aberration in their familial syndromic cancers. Effective treatments against *CDH1* inactivation would also benefit patients with sporadic diffuse gastric cancers since they often present with symptoms at a late stage.

1.3.1 The *CDH1* gene and it's Biological Functions

The tumour suppressor gene *CDH1* is implicated in hereditary and sporadic lobular breast cancers (Berx *et al.*, 1996; De Leeuw *et al.*, 1997; Berx and van Roy, 2009; Vos *et al.*, 1997; Semb and Christofori, 1998; Masciari *et al.*, 2007). The *CDH1* gene encodes the E-cadherin protein and is normally expressed in epithelial tissues, where it has also been identified as an invasion suppressor and loss of *CDH1* function has been implicated in breast cancer progression and metastasis (Berx *et al.*, 1995; Becker *et al.*, 1994; Christofori and Semb, 1999).

1.3.1.1 Cytoskeleton

The primary function of *CDH1* is cell-cell adhesion forming the adherens junction, maintaining the cytoskeleton and mediating molecular signals between cells. The function of the adherens complex is particularly important for cell structure and regulation because it interacts with cytoskeletal actins and microtubules. The cytoskeletal role of E-cadherin maintains healthy cellular viability and growth in epithelial tissues including cellular polarity. E-cadherin is not essential to cellular viability but loss in epithelial cells does lead to defects in cytoskeletal structure and proliferation. In addition to a central role in the adherens complex, E-cadherin is involved in many other cellular functions and thus *CDH1* is regarded as a highly pleiotropic gene.

1.3.1.2 Extracellular and Tumour Micro-Environment

As a transmembrane signaling protein E-cadherin also interacts with the extracellular environment and other cells, most notably forming tight junctions between cells. These junctions serve to both regulate movement of ion signals between cells and separate membrane proteins on the apical and basal surfaces of a cell, maintaining cell polarity. Thus E-cadherin is an important regulator of epithelial tissues by intercellular communication. It also has important roles in the extracellular matrix, including fibrin clot formation. The role of intercellular interactions and the tissue micro-environment are important themes in cancer research, being a potential mechanism for cancer progression and malignancy in a addition to it's potential for specifically targeting tumour cells.

1.3.1.3 Cell-Cell Adhesion and Signalling

The signals mediated by tight junctions are also passed on to intracellular signalling pathways and thus E-cadherin also has a role in maintaining cellular function and growth. One such example is the regulation of β -catenin which interacts with both the actin cytoskeleton and acts as a transcription factor via the WNT pathway. Similarly, the HIPPO and PI3K/AKT pathways are implicated in being mediated by E-cadherin, having roles in promoting cell survival, proliferation, and repressing apoptosis. E-cadherin shares several downstream pathways with signaling pathways such as integrins and thus indirectly interacts with them, particularly since feedback loops may occur in such pathways. Conversely, the multifaceted roles of E-cadherin have been shown with differing overexpression in ovarian cells promoting tumour growth, while it maintains healthy cellular functions in other cells.

1.3.2 *CDH1* as a Tumour (and Invasion) Suppressor

E-cadherin has key roles in maintaining cellular structure and regulating growth, consistent with *CDH1* being a tumour suppressor gene. Loss of *CDH1* in epithelial tissues leads to disrupted cell polarity, differentiation, and migration. E-cadherin loss has been identified as a recurrent driver tumour suppressor mutation in sporadic cancers of many tissues including breast, stomach, lung, colon, and pancreas tissue.

1.3.2.1 Breast Cancers and Invasion

E-cadherin loss in breast cancers has been shown to cause increased proliferation, lymph node invasion, and metastasis with poor cell-cell contact. Thus *CDH1* gene has also been implicated as an invasion suppressor, with a key role in the epithelial-mesenchymal transition (EMT), an established mechanism of cancer progression (Hanahan and Weinberg, 2011). The epithelial-mesenchymal transition is important during development and wound healing but such changes in cellular differentiation also occur in cancers. If *CDH1* is inactivated by mutation or DNA methylation (Berx *et al.*, 1996; Guilford, 1999; Machado *et al.*, 2001), it is likely that EMT will drive growth of E-cadherin deficient cancers (Berx and van Roy, 2009; Graziano *et al.*, 2003; Polyak and Weinberg, 2009). While loss of E-cadherin is not sufficient to cause EMT or tumourigenesis, it is an important step in this mechanism of tumour progression and a potential therapeutic intervention may therefore also impede cancer progression and have activity against advanced stage cancers.

1.3.3 Hereditary Diffuse Gastric Cancer and Lobular Breast Cancer

CDH1 loss of function mutations also causes familial cancers, including diffuse gastric cancer and lobular breast cancer (Guilford *et al.*, 1999; Graziano *et al.*, 2003; Guilford *et al.*, 2010; Oliveira *et al.*, 2009). Individuals carrying a null mutation in *CDH1* have a syndromic predisposition to early-onset these cancers, known as Hereditary Diffuse Gastric Cancer (HDGC) (Guilford *et al.*, 1998). Due to the loss of an allele, these individuals are prone to carcinogenic lesion in the breast and stomach when the other allele is inactivated, occurring much more frequently and thus younger than in individuals without a second functional allele of *CDH1*. The loss of the second allele is most often hypermethylation suppressing expression rather than mutation, although loss of heterozygosity may also occur. Therefore HDGC is an autosomal dominant cancer syndrome with incomplete penetrance. The “lifetime” (until age 80 years) risk for mutation carriers of diffuse gastric cancer is 70% in males and 56% in females. In addition, the lifetime risk of lobular breast cancer is 42% in female mutation carriers.

HDGC affects less than 1 in a million people globally (Ferlay *et al.*, 2015) and less than 1% of gastric cancers. However, HDGC is documented to affect several hundred families globally. E-cadherin mutations in the germline is implicated in 1-3% of gastric cancers presenting with a family history, varying between high and low incidence populations. E-cadherin is also mutated in 13% of sporadic gastric cancers.

While diagnostic testing for *CDH1* genotype has enabled more effective management of HDGC and improved patient outcomes, there are still limited options for clinical interventions. Individuals with a family history of HDGC are recommended to be tested for *CDH1* mutations in late adolescence and are offered prophylactic stomach surgery before the risk of developing cancers increases with age. Another option is annual endoscopic screening to diagnose early stage stomach cancers with surgical intervention once they are detected. However, these early stage cancers are difficult to detect and may be missed in regular screening. Thus patients carrying *CDH1* mutations either have surgical interventions with a significant impact on quality of life and risk of complications or remain at risk of developing advanced stage stomach cancers. Due to the lower mortality rate due to stomach cancers, there is increasing concerns among these HDGC families on the elevated risk of lobular breast cancers for women later in life.

The current clinical management of HDGC still has significant risks for patients

and therefore a greater understanding of the molecular and cellular function of *CDH1* is important for its role in these cancers. Such studies may lead to alternative treatment strategies such as pharmacological treatments with specificity against *CDH1* null cells, once they lose the second allele. While a loss of gene function cannot be targeted directly, designing a treatment with specificity against *CDH1* may also have activity in sporadic cancers in a range of epithelial cancers. Thus an effective treatment against *CDH1* mutant cancers would potentially have significant therapeutic and preventative applications in a large number of patients.

1.3.4 Somatic Mutations

1.3.4.1 Mutation Rate

Estimates for the prevalence of *CDH1* somatic mutations in sporadic cancers varies. The Cancer Gene Census detected 994 distinct mutations in 10,143 tumour samples (at a rate of 7.52%), COSMIC detected 632 distinct mutations in 43,865 tumour samples (at a rate of 1.71%), and the NCI60 detected mutations in 13.2% of 53 cancer cell lines. While there is no consensus on the prevalence of *CDH1* mutations, the vast variability of mutations is consistent with its role as a tumour suppressor and it has been found to be recurrently mutated in a wide range of cancers of epithelial tissues.

COSMIC reports *CDH1* mutations in 40 cancer tissue types including stomach (11.40% in N=1342), breast (10.29% in N=3343), large colon (2.87%), skin (2.83%), endometrial (2.81%), and bladder (1.9%) cancer. ICGC reports *CDH1* mutations in 29 cancer tissue types including skin (23.41% in N=598), breast (14.50% in N=1696), ovary (13.98%, N=93), and stomach (11.41% in N=289) cancer samples. *CDH1* mutations are reported at similar rates in breast and stomach cancer in other cancer genomics projects and studies across distinct populations. cBioPortal reports *CDH1* mutation prevalence in stomach cancer at 16.7% (Tokyo Univ., Kakiuchi, 2014, N=30), 15% (Pfizer/UHK, Wang, 2014, N=100), 14.1% (Tianjin Medical University, Chen, 2015, N=78), and 9.4% (TCGA, 2017 prov, N=393). cBioPortal also reports *CDH1* mutation prevalence in breast cancer at 12.7% (TCGA, 2017 prov, N=963) and 10.8% (METABRIC, 2012/2016, N=2051). The rare plasmacytoid bladder cancer subtype also has a high prevalence of *CDH1* mutations in COSMIC at a rate of 81.8% (N=33). These demonstrate that *CDH1* is important in many cancers and targeting *CDH1* may be widely applied against sporadic cancers in addition to hereditary cancers. However, some of these studies have focused on disease subgroups (such as lobular subtype or

estrogen receptor negative breast cancers) with poor patient outcomes which may have inflated the prevalence of *CDH1* mutations which are more common in some of these subtypes.

1.3.4.2 Co-occurring Mutations

Another concern is that *CDH1* mutations may co-occur with other known cancer driver genes such as highly prevalent tumour suppressor gene *TP53* or the proto-oncogene *PIK3CA*. cBioPortal reports the prevalence of the mutations in these genes at 10% for *CDH1*, 49% for *TP53*, 22% for *PIK3CA* in stomach cancer (TCGA, 2017 prov, N=393). There is no evidence of significant co-occurring mutations between *CDH1* and *PIK3CA* (mutex $p = 0.231$) but there is evidence for significant mutually exclusive mutations for *CDH1* (mutex $p = 0.002$) and *PIK3CA* (mutex $p = 0.004$) with *TP53*. cBioPortal also reports the prevalence of the mutations in these genes at 13% for *CDH1*, 32% for *TP53*, 36% for *PIK3CA* in breast cancer (TCGA, 2017 prov, N=3963). There is evidence of significant co-occurring mutations with *CDH1* and *PIK3CA* (mutex $p < 0.0001$) and evidence for significant mutually exclusive mutations for *CDH1* (mutex $p = 0.003$) and *PIK3CA* (mutex $p = 0.032$) with *TP53*.

These cancer driver mutations have distinct molecular features, leading to disease progression in distinct ways which is a concern for drug resistance when several mutations may accumulate, particularly for sporadic cancers where this is common. Targeting *CDH1* specifically is most suitable for hereditary cancers and combination therapies may be required for sporadic cancers. However, *CDH1* and *TP53* mutant cancers appear to be distinct pathways of tumour progression so the high impact of *TP53* mutation on cancer cells need not be considered for the purposes of studying *CDH1*.

1.3.5 Models of *CDH1* loss in cell lines

Previous work our research group has published used a model of homozygous *CDH1*^{-/-} null mutation in non-malignant MCF10A breast cells to show that loss of *CDH1* alone was not sufficient to induce EMT with compensatory changes in the expression of other cell adhesion genes occurring (Chen *et al.*, 2014). However, *CDH1* deficient cells did manifest changes in morphology, migration, and weaker cell adhesion (Chen *et al.*, 2014).

This *CDH1*^{-/-} MCF10A model has been used in a genome-wide screen of 18,120

genes using small interfering RNAs (siRNA) and a complementary drug screen using 4,057 compounds to identify synthetic lethal partners to E-cadherin (Telford *et al.*, 2015). One of the strongest candidate pathways identified by Telford *et al.* (2015) were the GPCR signalling cascades, which were highly enriched by Gene Ontology analysis of the candidate synthetic lethal partners the primary siRNAs screen. This was supported by validation with Pertussis toxin, known to target $G_{\alpha i}$ signalling (Clark, 2004), as were various candidate cytoskeletal pathways by inhibition of Janus kinase (JAK/STAT) and aurora kinase. The drug screen also produced candidates in histone deacetylase (HDAC) and phosphoinositide 3-kinase (PI3K) which were supported by validation and time course experiments.

1.4 Summary and Research Direction of Thesis

To address the needs and concerns raised by recent computational approaches to synthetic lethal discovery in cancer (Jerby-Arnon *et al.*, 2014; Lu *et al.*, 2015), we propose similar analysis using solely gene expression data which is widely available for a large number of samples in many different cancers. To firmly understand the limitations and implications of synthetic lethal predictions, we propose modelling and simulation of the statistical behaviour of synthetic lethal gene pairs in genomics data. Comparison of synthetic lethal gene candidates from public data analysis, predictions, and networks across datasets will address tissue-specificity concerns. Release of R codes used for simulation, prediction, and analysis will enable adoption of the methodology in the cancer research community and comparison to existing methods.

Synthetic lethality is an important genetic interaction to study fundamental cellular functions and exploit them for biomarkers and cancer treatment. While there are a wide range of experimental and computational approaches to synthetic lethal discovery, many are limited to particular applications, prone to false positives, and inconsistent across independent approaches to different genes of interest. Therefore synthetic lethal interactions are difficult to replicate or apply in the clinic. Computational approaches to synthetic lethality are not widely adopted by the cancer research community and experimental approaches cannot be combined to study synthetic lethality at a genome-wide scale. However, there is interest in synthetic lethal discovery in the community and the need for robust predictions of synthetic lethal interactions in cancer and human tissues. My thesis aims to develop such predictions with a focus on the example of E-cadherin to compare to the findings of Telford *et al.* (2015) and development of network

approaches to tissue specificity with the following bioinformatics and computational biology investigations:

- Simulate gene expression data, construct a statistical model for synthetic lethality, and measure performance of testing for synthetic lethal genes.
- Apply Synthetic lethal prediction to public genomics data
- Gene expression
- DNA copy number
- DNA methylation
- Somatic Mutation
- Select candidates for synthetic lethality with *CDH1* in breast cancer, compare to RNAi data for validation, and triage candidates for drug development against HDGC and sporadic breast cancers
- Release a synthetic lethal prediction methodology to the research community for wider application
- Construct and analyse genome-scale synthetic lethal networks, tissue specificity, or drug response using synthetic lethal predictions

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