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Review

Tumour heterogeneity and drug resistance: Personalising cancer medicine through functional genomics

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ABSTRACT

Intrinsic and acquired drug resistance leads to the eventual failure of cancer treatment regimens in the majority of advanced solid tumours. Understanding drug resistance mechanisms will prove vital in the future development of personalised therapeutic approaches. Functional genomics technologies may permit the discovery of predictive biomarkers by unravelling pathways involved in drug resistance and allow the systematic identification of novel therapeutic targets. Such technologies offer the opportunity to develop personalised treatments and diagnostic tools that may improve the survival and quality of life of patients with cancer. However, despite progress in biomarker and drug target discovery, inter-tumour and intra-tumour molecular heterogeneity will limit the effective treatment of this disease. Combining an improved understanding of cancer cell survival mechanisms associated with intra-tumour heterogeneity and drug resistance may allow the selection of patients for specific treatment regimens that will maximise benefit, limit the acquisition of drug resistance and lessen the impact of deleterious side effects.

Contents

1. Introduction

Most advanced solid tumours eventually develop drug resistance, limiting the effective management of cancer in the clinical setting [1,2]. Despite the many new targeted therapeutic agents that are now being used clinically, acquired and intrinsic drug resistance remains a major clinical problem [3]. Significant intra-tumour genetic heterogeneity [4,5] is likely to limit the efficacy of targeted therapies prior to the acquisition of drug resistance.

Several models of cancer drug resistance have been proposed. Firstly, evolutionary acquisition of drug resistance may occur. Mechanisms such as gene mutations may allow a subpopulation of cancer cells to gain a selective advantage in the face of a selection pressure and proceed to propagate drug-resistant clones [6]. In the

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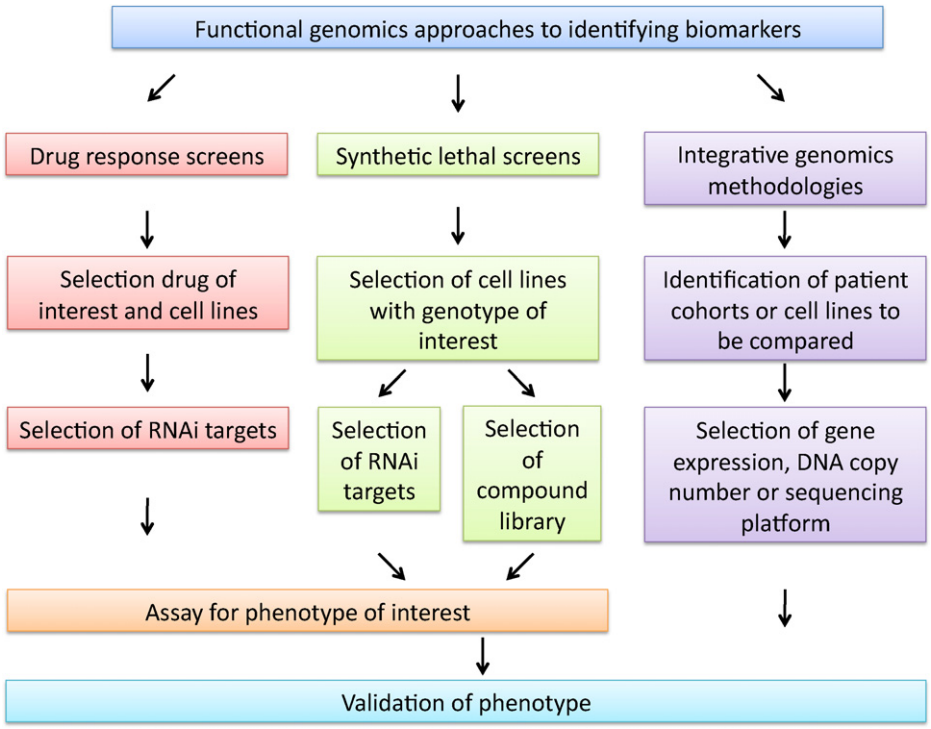

Fig. 1. Functional genomics approaches to identifying biomarkers. Three main approaches are discussed in this article, loss of function RNAi screens, synthetic lethality screens and integrative genomics methodologies using gene expression, DNA copy number and sequencing platforms.

cancer stem-cell model of drug resistance, drug resistance is mediated by tumour stem cells. Following drug treatment, daughter cancer cells are eradicated, but cancer stem cells may be able to survive due to their ability to better tolerate stresses such as DNA damage [7]. The cancer stem cells are then able to mediate regrowth of the tumour through the generation of new daughter cells. Finally, cancer cells may be intrinsically resistant to drug treatment. Alterations in gene dosage and signalling pathways within the cancer cell population may render them intrinsically more drug resistance and hardier compared to normal non-cancerous cells. Further understanding of drug resistance and cancer cell survival pathways is essential in order to develop predictive biomarkers that allow for the personalisation of cancer therapy to overcome the problem of intrinsic drug resistance and minimise the effects of acquired drug resistance. Maximising patient benefit and minimising drug costs may be enhanced through the development of predictive biomarkers in order to define subsets of patients that are most likely to derive benefit from particular treatments. Importantly, subsets of patients that are unlikely to respond to a particular drug treatment may also be defined through these approaches that may enable such patient cohorts to be prioritised for clinical trials.

In loss of function screens, RNAi technology is commonly used to enable short- or long-term gene silencing in cultured cell lines in the laboratory [8], to assess the phenotypic effects of each gene. Such technology has been effectively exploited to begin the systematic dissection of cancer drug sensitivity mechanisms. This review focuses on developments in our understanding of pathways mediating drug resistance in cancer and the identification of new drug therapy targets using such RNAi-based functional genomics approaches. We discuss the findings of loss of function genetic screens and integrative approaches derived from tumour gene expression, DNA copy number and DNA sequencing datasets (Fig. 1).

A common thread linking these studies is that response to therapy is influenced by a large number of different genes, and cancers demonstrate a diverse range of dependencies on different pathways for survival. The targeting of single molecular pathways in advanced solid tumours is likely to fail due to the presence of intra-tumour genetic heterogeneity that will foster the acquisition of drug resistance. In this review we discuss the implications of tumour heterogeneity in biomarker discovery and personalised medicine and suggest that a detailed understanding of both drug resistance and intra-tumour heterogeneity will be necessary to develop the next generation of personalised therapeutics to limit treatment failure.

2. RNAi screens to identify pathways regulating drug resistance

Short hairpin RNA (shRNA) or small interfering RNA (siRNA) technologies are commonly used in the RNAi screens presented in this article. shRNA is cleaved into siRNA by the Dicer enzyme and the siRNA antisense strand acts as a template to target the RISC (RNA-induced silencing complex) enzyme complex to single stranded mRNA for degradation [9]. A complementary match of siRNA with mRNA results in cleavage of the mRNA whilst a partial match of siRNA and mRNA results in translational block and is similar to microRNA (miRNA) processing. Both processes result in a decrease in protein expression [10]. shRNA can be expressed in cells using lentiviral expression vectors allowing for the propagation of gene silencing in subsequent daughter cells and thus long-term gene silencing in the cell population [11]. siRNAs on the other hand, are commonly introduced directly into a cell using cationic lipid transfection agents that allow cells to take up the nucleic acids [12].

Many loss of function RNAi screens have successfully revealed pathways that are involved in the *in vitro* acquisition of drug resistance. These studies have focused on both traditional cytotoxics and newer targeted therapies. Such approaches may present the first step in a discovery strategy to identify candidate biomarkers to predict tumour drug sensitivity or identify novel

Table 1
RNAi screens for regulators of drug response.

Aim of screen	Screening method	Number of targets	Number of validated candidates	Potential *in vivo* markers	Reference
AKT inhibitor sensitivity	siRNA	507 kinases	6		Morgan-Lappe et al. [29]
ATRA sensitivity	shRNA	8500	26		Hattori et al. [30]
Cisplatin sensitivity	siRNA	Whole genome	53	BRCA1/2	Bartz et al. [21]
Gemcitabine sensitivity	siRNA	2400	Conventional validation not done	CHEK1	Bartz et al. [21]
Gemcitabine sensitivity	siRNA	645 kinases	83	CHEK1	Giroux et al. [26]
Paclitaxel sensitivity	siRNA	Whole genome	87	ACRBP	Whitehurst et al. [15]
Paclitaxel/cisplatin/doxorubicin/ 5-FU sensitivity	siRNA	779 kinases and 50 ceramidome	25 (paclitaxel)	CERT	Swanton et al. [17]/ Lee et al. [20]
PARP inhibitor sensitivity	siRNA	779 kinases	6	CDK5	Turner et al. [34]
R3200, acyl sulfonamide derivative, sensitivity	shRNA	8000	14		Mullenders et al. [28]
Tamoxifen sensitivity	siRNA/shRNA	779 kinases/Whole genome	3/23	NF1/Resistance metagene (BAP1, RARG, PTEN, SMC3, NF1, NIPBL,UBA3, and CDK10) and Sensitivity metagene (EDF1, KRAS, RAF1, TMPRSS2, TPM4, and PDPK1)	Iorns et al. [32]/ Mendes-Pereira et al. [33]
Trastuzumab sensitivity	shRNA	8000	1	PIK3CA, PTEN	Berns et al. [31]

targets that may allow for the enhancement of drug efficacy through the discovery of drug sensitisation pathways.

2.1. RNAi screens for regulators of cytotoxic sensitivity

Many studies have focused on identifying pathways that influence the response of cancer cells to commonly used cytotoxics such as paclitaxel, cisplatin and gemcitabine (Table 1).

Paclitaxel treatment arrests cells in mitosis [13] where they subsequently undergo cell death during arrest or following mitotic slippage into a multinucleated interphase [14]. In a genome-wide analysis of genes whose loss of function mediate paclitaxel sensitisation in a human non-small-cell lung cancer (NSCLC) cell line, NCI-H1155, Whitehurst and colleagues identified 87 genes that, when silenced enhanced the cytotoxicity of paclitaxel [15]. Acrosin-binding Protein (ACRBP) was one candidate gene identified that resulted in paclitaxel sensitivity when silenced. In a follow-up study, high ACRBP expression was found to correlate with poorer prognosis of cancer patients [16]. The main overlapping pathways shared by the 87 genes were genes that formed components of the proteasome and genes involved in forming the gamma-tubulin ring complex. In another investigation of paclitaxel drug response pathways, a kinome (779 genes) and ceramidome (50 genes) siRNA screen for regulators of multidrug resistance in HCT116 (colorectal cancer (CRC)), MDA-MB-231 (breast cancer) and A549 (non-small cell lung cancer) cell lines, identified pathways involved in maintaining chromosomal stability were associated with taxane resistance [17] suggesting that tumour Chromosomal Instability (CIN, see Section 5) status in advance of therapy might determine paclitaxel treatment response [18]. Further examining the link between CIN and intrinsic taxane resistance, our laboratory demonstrated that CIN cancer cells overexpress a set of genes termed the microtubule-stabilising (MTS) gene signature [19], enabling the cells to survive the down-regulation of these genes following MTS exposure.

The ceramide transporter protein CERT was identified in the same kinome and ceramidome screen described in the previous paragraph to promote multidrug sensitivity to paclitaxel, doxorubicin, cisplatin and 5-FU (fluorouracil) when silenced by RNAi [17], indicating another pathway that could be targeted to limit multidrug resistance in cancer. Further investigation of this gene revealed that CERT depletion results in LAMP2 (lysosomal-associated membrane protein 2) dependent multidrug sensitisation that is associated with alterations in autophagosome-lysosomal flux. Supporting the utility of genes identified through RNAi screens to be used as biomarkers, it was found that high CERT expression is associated with HER2+ status in breast cancer, poorer breast cancer specific survival in a cohort of 356 patients and shorter relapse-free survival in a meta-analysis of gene expression data across 1659 patients [20].

A genome-wide siRNA screen to identify cisplatin (a platinum based drug) sensitisers in TP53 deficient HeLa cells [21] identified that pathways involved in DNA-damage repair regulate cisplatin sensitivity (consistent with the DNA damage-inducing mechanism of action of cisplatin). Many of these genes are members of the BRCA DNA damage repair pathway (BRCA1, BARD1, BRCA2, and RAD51) concordant with clinical evidence that mutations in BRCA may result in better response to platinum-based drugs [22–24]. In agreement with the results of the previous screen, RAD51 was also identified in an RNAi screen to establish cisplatin sensitisers in *Caenorhabditis elegans* germ cells [25].

Two smaller scale RNAi screens were performed to identify gemcitabine sensitisers using 2400 siRNAs in HeLa cells [21] and 645 siRNAs targeting kinases in the pancreatic cancer cell line MiaPaCa-2 [26]. CHEK1 overlapped in both screens as a candidate gene that when silenced enhanced gemcitabine action. Analysis of breast cancer microarray datasets reveals that high CHEK1 expression is associated with poorer prognosis in cancer [27]. The lack of overlap of candidate genes between the two screens may represent the different sets of siRNA used and different genetic backgrounds of the cell lines.

Finally, an shRNA screen targeting 8000 genes was performed to identify genes that influenced sensitivity to R3200, a new acyl sulfonamide derivative with an unknown mechanism of action [28] in the HCT116 CRC cell line. RBX1 and DDB1 that form part of an E3 ubiquitin ligase complex, were identified as genes that caused resistance to R3200 when silenced. Such approaches may lead to the identification of potential biomarkers for the use of drugs with unknown mechanisms of action that may augment the ability to demonstrate efficacy in molecularly pre-defined tumour cohorts.

2.2. Targeted therapies

RNAi screening approaches have allowed us to elucidate mechanisms of acquired resistance to targeted therapeutics with the potential to further refine patient selection based on biological markers. Screens have been performed to identify regulators of

drug response to agents such as AKT inhibitors [29], all-trans retinoic acid (ATRA) [30], Trastuzumab [31], tamoxifen [32,33] and poly (ADP-ribose) polymerase (PARP) inhibitors [33] (Table 1).

An siRNA screen targeting 507 kinases was performed to identify sensitisers of the AKT ATP-competitive inhibitor, A-443654, in the PTEN-deficient renal cell carcinoma cell line, 786-O [29]. CSNK1G3, IPMK, MAPKAPK2, RSKB, MAP4K2 and MPP1 were identified as enhancers of cytotoxicity when silenced.

ATRA is commonly used in the treatment of Acute Promyelocytic Leukaemia (APL) as APL is associated with translocations involving the retinoic acid receptor alpha. An shRNA screen to identify regulators of ATRA-induced differentiation and growth arrest in the NB4 APL cell line identified 26 genes that resulted in ATRA resistance. These genes mediated diverse functions including gene expression, intracellular signalling, cell death control, stress responses, and metabolic regulation [30].

Trastuzumab is a monoclonal chimeric mouse–human antibody that acts by targeting the HER2 receptor [35] that is overexpressed in a subset of breast cancer. PTEN silencing was found to induce Trastuzumab resistance in an RNAi screen in the HER2+ breast cancer cell line, BT474 [31]. PTEN is a negative regulator of PIK3CA and further retrospective examination of breast tumour samples demonstrated that activating PIK3CA mutation or loss of PTEN were associated with poorer outcome following Trastuzumab treatment compared to patients with cancers that did not harbour activated PI3-Kinase pathway signalling.

Tamoxifen is a mixed agonist/antagonist of the oestrogen receptor (ER) [36] that has been shown to benefit women with ER-positive breast cancer in the adjuvant and metastatic setting [37,38]. In an analysis of determinants of tamoxifen response, an ER-positive breast cancer cell line, MCF7, was screened against 779 siRNAs targeting kinases [32]. Three validated candidate genes that caused resistance to tamoxifen when silenced were obtained – CDK10, CRK7 and MAP2K7. Assessment of CDK10 levels in two ER-positive breast cancer clinical datasets (87 patients and 38 patients respectively) revealed that low CDK10 levels were associated with poorer overall survival and a higher probability of cancer relapse. In a follow-up whole genome shRNA screen by the same senior authors to identify genes affecting sensitivity to tamoxifen [33], 12 validated genes causing resistance and 11 validated genes causing sensitivity were identified. Gene expression microarray data and time to distant relapse data were available for five patient datasets, with a total number of 432 patients. Using data from this screen and the previous tamoxifen screen, metagenes for resistance and sensitivity that correlated with clinical outcome were obtained. The metagene predicting resistance comprised of eight validated genes (BAP1, RARG, PTEN, SMC3, NF1, NIPBL, UBA3 and CDK10) and the metagene predicting sensitivity comprised of six validated genes (EDF1, KRAS, RAF1, TMPRSS2, TPM4, and PDPK1).

PARP inhibitors result in the generation of DNA damage and this is particularly toxic to cells with deficient DNA homologous recombination such as BRCA null cancer cells [39]. In a kinome siRNA screen for regulators of sensitivity to PARP inhibitors, CDK5 was identified to mediate PARP inhibitor sensitivity when silenced [34]. Analysis of genome copy number and gene expression data set revealed that genomic loss of CDK5 with resulting reduction in gene expression occurred in 5.5% of breast cancer patients. This was felt to potentially identify a subset of patients who may benefit from treatment with PARP inhibitors.

3. Synthetic lethal screens to identify novel drug targets and biomarkers

Screens can be performed to identify genes whose loss of function results in synthetic lethality or sickness, where the combination of loss of function of a gene within a particular genotype of interest results in a decrease in cell viability compared to an isogenic cancer cell without that genotype [40–42]. The identification of such synthetic lethal survival pathways may lead to novel targeted therapies for subsets of cancers with the promise of less severe side effects to surrounding normal tissue where disruption of the pathway does not result in lethality.

These studies can be performed in isogenic cell lines, with identical genetic backgrounds apart from the single genotype of interest. A loss of function screening approach allows for the disruption and identification of the synthetic lethal pathway.

3.1. RNAi synthetic lethal screens in isogenic cell lines

RNAi screens have been reported to identify genes that are regulators of synthetic lethality in cancer cells (Table 2). Genes or pathways that regulate synthetic lethality may then be developed as new therapeutic targets. The majority of published synthetic lethal screens have not been genome-wide screens. However a genome-wide RNAi screen using siRNA has been performed in isogenic HCT116 CRC cell lines with one cell line being deficient for the tumour suppressor TP53 [43], required for cell cycle arrest, apoptosis and senescence [44]. The two isogenic cell lines were fluorescently labelled with RFP or GFP respectively and seeded in an equal ratio and transfected with siRNA in the same tissue culture microplate well. Three candidate genes, UNRIP, MASTL and KIAA1344 were identified to selectively target the p53 null cell line. The authors further investigated UNRIP, which gave the strongest synthetic lethal phenotype, and demonstrated that p53 null cells showed greater dependence on small nucleolar ribonucleoprotein (snoRNP) assembly, a process that is attenuated through UNRIP depletion by RNAi.

There has been a greater amount of published data for smaller scale RNAi screens for synthetic lethality. One such screen has been a 100-strong shRNA screen (targeting 88 unique kinases) for survival regulators in VHL null isogenic cancer cell lines [45]. VHL is frequently biallelically inactivated in clear cell renal carcinoma. Five candidate genes were identified that preferentially targeted VHL−/− cells in both 786-0 and RCC4 isogenic cell lines. CDK6,

Table 2
Screens for synthetic lethality.

Aim of screen	Screening method	Number of targets	Number of validated candidates	Potential *in vivo* markers	Reference
BRCA2 synthetic lethality	Anti-cancer compounds	1258	3		Evers et al. [54]
EGFR synthetic lethality	Anti-cancer compounds	1990	8		Trembath et al. [49]
KRAS synthetic lethality	shRNA	2500	1		Wang et al. [44]
KRAS synthetic lethality	shRNA	100	3	WT1	Vicent et al. [45]
KRAS synthetic lethality	shRNA	Genome-wide	50	COPS3, CDC16	Luo et al. [40]
KRAS synthetic lethality	siRNA	4000	3		Sarthy et al. [47]
PTEN synthetic lethality	Anti-cancer compounds	138,758	2		Li et al. [53]
TP53 synthetic lethality	siRNA	Genome-wide	3		Krastev et al. [43]
VHL synthetic lethality	shRNA	88	5		Bommi-Reddy et al. [45]

MET, and MEK1 were validated using lower shRNA titres and siRNA sequences to preferentially inhibit the viability of VHL−/− cells.

K-ras activating mutations are commonly found in solid tumours. A Mitogen activated protein kinase (MAPK) cascade is activated following K-ras signalling, whose downstream targets include transcription targets regulating processes such as cell cycle transition. K-ras also activates the PI3-Kinase/Akt/pathway, contributing to cell growth, proliferation, and survival [46]. Synthetic lethal screens have attempted to identify regulators that are required for the survival of cancer cells harbouring activating K-ras mutations.

Two shRNA screens revealed a role for pathways regulating Epithelial–Mesenchymal Transition (EMT) in the survival of cancer cells with activated mutant K-ras. Wang and colleagues performed an shRNA screen involving 2500 genes including the majority of known protein kinases and cancer-related genes using isogenic HCT116 CRC cell lines with either mutated or wild-type K-ras [47]. Snail2, a protein involved in EMT transition, was the most potent candidate gene identified to induce lethality in cells with mutated K-ras. In another screen, 100 genes were targeted by shRNA [48] in mouse embryonic fibroblasts (MEFs) engineered to either express oncogenic or wild-type K-ras. These 100 target genes were selected based on a K-ras and K-ras correlated transcription factors gene signature. Three genes, RAC1, PHB2 and WT1 were negatively selected in cells with mutated K-ras. Further investigation of WT1 depletion in human NSCLC cell lines revealed preferential lethality in cells with K-ras mutations. Analysis of gene expression data for a cohort of lung cancer patients revealed that for tumours that expressed an activated K-ras gene signature, higher expression of a WT1 gene signature was associated with poor prognosis whilst lower expression of the WT1 signature was associated with good prognosis. In tumours that did not express the activated K-ras signature, the WT1 gene signature did not confer any prognostic value. As WT1 regulates the EMT process [49], these two screens demonstrate that RNAi screens can identify common pathways that regulate synthetic lethality.

Additional screens for K-ras synthetic lethality include a genome-wide shRNA screen in a DLD1 CRC isogenic K-ras model. Fifty candidate genes validated in two different isogenic model cell lines (DLD1 and HCT116) as being preferentially lethal towards K-ras deficient cells when silenced. The authors identified that a high proportion of the synthetic lethal genes identified were mitotic regulators. Microarray gene expression data of a lung cancer cohort was analysed and low expression of two of the genes identified – COPS3 and CDC16 were found to be associated with enhanced survival in patients with tumours demonstrating an activated Ras signature. However in patients with tumours with a non-activated Ras signature, expression of these genes did not appear to confer a prognostic value.

In an siRNA screen targeting 4000 genes including protein kinases, G protein-coupled receptors, ubiquitin E3 ligases, transporters, ion channels, and peptidases in an isogenic CRC model with activated K-ras [50], a different set of synthetic lethal regulators were identified. Survivin, CDK1 and C20ORF18 were identified as the top candidates to induce preferential lethality in a DLD1 CRC cell line with activated K-ras. The identification of CDK1 supports the findings of the previously discussed whole-genome screen that mitotic regulators may play a role in K-ras synthetic lethality. Interestingly there appeared to be a lack of overlap of synthetic lethal regulators between the studies done using DLD1 cell lines and the studies using HCT116 or MEF lines suggesting that the results may be cell line-specific.

3.2. Anti-cancer compound synthetic lethal screens in isogenic cell lines

Several compound screens to identify drugs that may target cell lines with distinct genotypic backgrounds have been performed (Table 2). In a compound screen performed in an isogenic K-ras transformed human prancreatic ductal epithelial cell lines, screening approximately 3200 compounds revealed candidates that appeared to preferentially target the cell line with activated K-ras [51]. Unfortunately, the mechanism of action of the top compound identified was unknown, demonstrating that although compound screens in isogenic cell lines may reveal synthetic lethal dependencies, the lack of systematic disruption of molecular pathways, typified by genome-wide RNAi screens, makes it challenging to interpret the results.

Similarly, in a small molecule inhibitor screen to target cells expressing mutant EGFR [52] using an isogenic human glioblastoma cell line model with the deletion mutation EGFRvIII, one compound, NSC-154829 was identified. Its mechanism of action was undefined although the compound possessed a purine-like structural component.

However, compound screens using isogenic cell lines with mutated PTEN and BRCA2 have allowed for the identification of compounds with known mechanisms of action that resulted in synthetic lethality. PTEN, a tumour suppressor gene frequently mutated in cancer [53], regulates PI3K/Akt pathway activity and cellular processes such as apoptosis and cell cycle progression [54]. Hereditary BRCA2 mutations are associated with increased susceptibility to breast and ovarian cancers due to impairment of DNA damage repair [55]. In a high throughout screen involving 138,758 drug compounds for regulators of PTEN sensitivity involving isogenic HCT116 CRC cell lines, 2 compounds were identified that resulted in greater lethality in the PTEN null cells [56]. The authors further investigated one of their candidate compounds, CID1340132, and found that it induced an increased in the formation of gamma-H2AX, indicative of DNA damage in the form of double stranded breaks. For BRCA2, a high-throughput pharmaceutical screen using the LOPAC1280 compound library (1258 compounds) in isogenic BRCA2 deficient mouse mammary tumour cells was performed [57]. Three alkylator drugs (chlorambucil, melphalan, and nimustine) were identified to cause selective toxicity in BRCA2 null cells [58]. These drugs appeared to result in a greater specific activity compared to cisplatin and the PARP inhibitor olaparib (AZD2281), with synergistic interactions with olaparib reported.

4. Integrative genomics methodologies

Gene expression and DNA copy number and sequencing studies integrated with functional analyses have allowed the identification of sets of genes that can predict response to a particular form of chemotherapy. This approach allows for the identification of multiple genes or genomic regions that may be implicated in tumour response to therapy and clinical outcome.

In an extensive investigation of gene expression profiles across breast carcinomas [59], a predictive analysis of microarrays was performed and 75 genes identified as being differentially expressed in tumours with early recurrence after anthracycline treatment. Chromosomal region 8q22 was significantly enriched in probes associated with metastatic recurrence (12 genes). Further investigation by single nucleotide polymorphism (SNP) array analysis of DNA copy number in 50 breast cancers revealed that 8q22 amplification was observed and expression of the 8q22 genes correlated with DNA copy number. siRNA silencing of LAPTM4B, identified through this analysis, resulted in sensitisation to anthracyclines and conversely overexpression of this gene resulted in resistance.

Recent cancer sequencing studies have demonstrated profound inter-tumour heterogeneity in somatic mutation status. This is compounded by emerging evidence for intra-tumour heterogeneity in solid tumours. Both processes are likely to have implications

for biomarker discovery and validation as well as the acquisition of drug resistance clones during therapy [60]. Such observations support the need to rapidly identify the genomic basis for such heterogeneity across different tumour types in order to develop therapeutics that may efficiently limit intra-tumour diversity and Darwinian fitness [6]. Integrative tumour functional genomics approaches will provide the tools necessary to decipher such complex disease processes.

5. Targeting Chromosomal Instability to overcome drug resistance and tumour heterogeneity

Genomic instability mechanisms provide a means to acquire genetic heterogeneity within the cancer cell population. One such mechanism of genomic instability occurs through Chromosomal Instability (CIN), which describes an increased rate of ongoing numerical and structural chromosomal changes within cells [61,62]. Mechanisms ensuring the fidelity of proper chromosome segregation are compromised during cellular division in CIN+ cells and are maintained in subsequent generations of cell division, resulting in the generation and propagation of aneuploid cells. Aneuploidy is permissive for the rapid evolution of multidrug resistance [63] and is associated with intrinsic taxane [17] and acquired drug resistance [64]. Targeting pathways responsible for the generation or survival of CIN+ cells may reduce the acquisition of multidrug resistance in cancer.

Various studies have provided evidence that the CIN/aneuploid phenotype may be exploitable to better target these cancer cells. Roschke and colleagues have demonstrated the existence of anticancer compounds that may specifically target karyotypically complex cancer cells [65] indicating that it may be possible to target CIN+ cancer cells specifically. Experiments performed in yeast model systems have enabled us to gain further insight into the adaptations acquired by aneuploid cells in order to tolerate aneuploidy. A genome-wide yeast deletion mutant screen revealed that polyploid yeast are dependent for survival upon increased expression of genes involved in pathways such as sister chromatid cohesion and mitotic spindle function [66]. Torres and colleagues demonstrated using an analysis of haploid and wild-type yeast strains that strains of aneuploid yeast show delayed cell cycle transition, an increase in glucose uptake, and a gene expression pattern characteristic of the environmental stress response [67].

The finding of a gene signature termed the CIN70 gene signature [68], that acts as a surrogate measure for CIN [69], suggests that there are common pathways that are deregulated across CIN+ tumours of various types. Key regulators of chromosome segregation were present in the gene signature, including *MAD2*, *AURKA*, *AURKB*, *CDC20*, and *ZWINT* suggesting that CIN cancer cells may have a greater dependency on pathways involved in chromosome duplication and segregation, similar to that observed in experiments involving polyploid yeast [66]. Human homologs of genes that were required for the survival of polyploid yeast were found to be present in the CIN70 signature, including *AURKB*, *ch-TOG*, *ESPL1*, *FEN1* and *RAD21*.

These studies indicate that CIN cells require adaptations to tolerate the CIN state and that there are pathways that are commonly deregulated in CIN+ tumour cells of differing tissue origins. This suggests the intriguing possibility that karyotypic instability may be specifically targeted in human cancer for death, whilst limiting drug resistance and cytotoxicity to surrounding normal non-CIN tissue.

5.1. CIN as a prognostic biomarker

Defining levels of CIN in cancer may be useful to stratify patients for effective treatment and to provide prognostic biomarkers. It is challenging to quantify CIN, especially *in vivo* as it requires the measurement of rate of change of whole chromosome number or structure across a cell population. Instead, proxy methods of scoring CIN are used; static measures that take a "snapshot" at a certain point in time that measure the number of chromosomes, chromosome complexity and genetic diversity in a supposedly clonal population.

Flow cytometry is commonly used to measure the DNA content of cancer cells and by extension, estimate the ploidy index [70]. The stemline scatter index (SSI) derived by flow cytometry experiments provides a clonal heterogeneity score that allows for the classification of tumours into diploid and aneuploid stable or unstable states [71]. Other established methods of scoring CIN include labour intensive methods such spectral karyotyping (SKY) and centromeric FISH. SKY allows scoring of modal chromosomal number, structural or numerical complexity and heterogeneity of chromosome number between cells [62]. Centromeric FISH allows the counting of the numbers of chromosomes, permitting a description of aneuploidy and chromosomal numerical heterogeneity.

Vast amounts of aCGH, SNP and expression microarray data are available on various platforms, which can be used to derive surrogate measures of aneuploidy or CIN [62,69,69,71–73]. Recently developed algorithms for the analysis of biallelic SNP arrays allow for the estimation of ploidy levels, loss of heterozygosity (LOH) events and the degree of chromosomal structural instability and the location of aberrant chromosomal regions [74,75].

Increasing evidence suggests that CIN is associated with poor prognosis in solid tumours [68,70,76]. A relationship of chromosomal instability as scored by SSI with prognostic breast cancer gene expression signatures such as MammaPrint and Oncotype DX has been reported, suggesting that prognostic gene expression signatures are linked to chromosomal instability [77]. It has been suggested that adverse outcome associated with CIN may be related to increased tumour cell heterogeneity driving the ability of tumours to adapt to environmental stresses [78–80]. However, whilst an increased level of CIN may be beneficial in driving adaptation, more extreme levels of CIN and concomitant severe genetic disruption may compromise fitness, suggesting that an "optimal" level of CIN for carcinogenesis may exist [60,69,78,81].

Recently, our laboratory has demonstrated that there exists a non-monotonic relationship between CIN and clinical outcome for a variety of cancer types [69]. According to this model, tumours with an extreme level of CIN, determined by CIN70 gene expression aCGH-based measures of structural chromosomal complexity or direct FISH quantification of numerical CIN, had a poorer survival compared to patients with less extreme CIN [60,69]. This may be caused by higher degrees of on-going chromosomal alterations that are not tolerated by tumour cells analogous to bacterial population genetics models of mutational meltdown, where random genetic drift overwhelms the ability of natural selection to eradicate incoming deleterious mutations and leads to eventual extinction of the population [82]. Furthermore, animal models of CIN demonstrate that excessive levels of CIN can act to suppress tumour formation [83]. If such an association of extreme CIN with improved outcome is demonstrated in prospective analyses of larger patient cohorts, this suggests that efforts to accelerate CIN in tumours may prove beneficial. This may be achieved by using drugs that target pathways regulating chromosome segregation, including drugs that interfere with mitosis, or using drugs that increase genomic instability, such as DNA damaging agents. Further identification of the molecular mechanisms that help to maintain CIN *in vivo* may also contribute to the development of new drugs that can alter the rate of CIN within cancer cells.

6. Conclusion

The functional genomics studies discussed here have identified genes regulating drug response *in vitro* that may provide the first step in the discovery of predictive biomarkers to stratify patients for distinct personalised therapeutic approaches. Taken together, these studies demonstrate that drug resistance is a complex process that likely involves a myriad of genes regulating response to drugs with diverse mechanisms of action. Although these findings may allow for the development of predictive biomarkers of treatment efficacy, the capacity for intra-tumour heterogeneity to initiate treatment failure should be considered [10]. One pattern of genome instability fostering intra-tumour heterogeneity, CIN, may be permissive for the acquisition of multidrug resistance. Targeting CIN may limit drug resistance in the clinical setting by crippling the ability of cancers to maintain genetic heterogeneity leading to profound fluxes in gene expression that may support cancer cell phenotypic diversity.

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Conflict of interest

The authors declare no conflicts of interest.

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