Copy number networks to guide combinatorial therapy of cancer and proliferative disorders

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ABSTRACT

Interaction networks can be charted by seeking gene pairs that are amplified and/or deleted in tandem, even when located at a distance on the genome. Our experience with radiation hybrid (RH) panels, a library of cell clones that have been used for genetic mapping, have shown this tool can pinpoint statistically significant patterns of co-inherited gene pairs. In fact, we were able to identify gene pairs specifically associated with the mechanism of cell survival at single gene resolution. Further, the RH network can be used to provide single gene specificity for cancer networks constructed from correlated copy number alterations (CNAs). In a survival network for glioblastoma, we found that the epidermal growth factor receptor (EGFR) oncogene interacted with 46 genes. Of these genes, ten (22%) happened to be targets for existing drugs. Here, we highlight the potential of CNA networks to guide combinatorial drug treatment in cancer, autoimmunity and atherosclerosis.

Introduction

New drug discovery is confronted by rising costs and diminishing success rates. In response to these obstacles, genome and network data have been used to reposition drugs outside their usual domain or to design novel drug combinations. The networks used in these efforts typically consist of transcriptional co-expression networks (Ahn et al., 2009; Zhang and Horvath, 2005), protein-protein interaction networks (Geva and Sharan, 2011; Giot et al., 2003; Venkatesan et al., 2009; Vidal et al., 2011; Yu et al., 2008) or, in non-vertebrate model organisms, genetic interactions (Costanzo et al., 2010; Lehner et al., 2006). However, these networks have many false-positives and false-negatives (Bruckner et al., 2009; Mackay et al., 2007) and also suffer from bias (Coulomb et al., 2005). Less effort has been devoted to constructing mammalian

networks at the level of the gene. Here, we focus on genetic interactions in mammalian cells identified from correlated patterns of unlinked copy number alterations (CNAs). These networks represent an opportunity for the design of novel treatments and are particularly relevant to antiproliferative therapies in disorders such as cancer and autoimmunity.

A diminishing drug pipeline

Small organic molecules continue to be the mainstay of medical therapies, though prominent niche roles are being taken by macromolecules, such as interfering RNA, gene therapy and therapeutic antibodies. Regardless of modality, it is increasingly difficult to gain approval for new drugs, leading to blocked therapeutic pipelines (Csermely et al., 2013; Gupta et al., 2013; Pujol et al., 2010; Zou et al., 2013). New drugs can fail at multiple steps in the testing process, often because of unexpected safety or toxicological concerns. Another relevant factor is the enormous development costs of new drugs. Eroom's Law (Moore's Law backwards), observes that the number of therapies developed per research dollar has halved every nine years for decades (Scannell et al., 2012; Wobbe, 2008).

Using genome data to replenish the pipeline by drug repositioning

To open up the pipeline there is growing interest in using genomic and network data to design new drug therapies and minimize side effects. For example, one recent study combined transcript profiling data from many studies to identify CD44 gene expression as strongly correlated with type II diabetes mellitus (Kodama et al., 2012). Introducing CD44 deficiency into mouse models blunted the effects of diabetes, suggesting that targeting this molecule will have useful therapeutic effects.

In addition to identifying new drug targets, networks can be used to redeploy drugs from other disorders (Gupta et al., 2013; Zou et al., 2013). This approach is called drug repositioning or repurposing. One strategy uses multidimensional readouts of drug exposed cells to construct networks of drug-drug similarities. Modules of interconnected drugs can then predict compounds that will have efficacy in novel settings (Gottlieb et al., 2011; Iorio et al., 2010; Iskar et al., 2013). Drug repositioning has also been explored by evaluating protein interactions common to different drugs, constructing personalized drug networks from genome-wide association studies, and using drug side effects to suggest novel therapeutic areas (Csermely et al., 2013; Pujol et al., 2010).

The small world properties of networks expedite combination therapies

Biological networks display "small world" properties, whereby any two genes are separated by only a small number of links (Watts and Strogatz, 1998). If each gene interacts with 30 others, a gene connects with 900 genes in two steps (i.e. 30^2), and with all genes within three steps (30^3 > 20,000 genes). In fact, the average path length in biological networks (the number of links between any two genes) varies between roughly 2 to 4 interactions. Thus, nearly all genes are linked within a short number of steps to all other genes (Albert, 2005; Albert and Barabási, 2002; Tsaparas et al., 2006; Vidal et al., 2011; Xu et al., 2011; Zou et al., 2012).

There are nearly 3,000 Food and Drug Administration (FDA) approved drugs (http://www.fda.gov). After accounting for overlapping targets, it is estimated that these drugs affect approximately 1,000 different gene products (Overington et al., 2006), meaning that approximately 1 in 20 genes is a target for an FDA approved drug. Within one step, each gene will thus interact with one to two drug targets, and within two steps, 45 targets. Therefore, even if no drug is available for a disease gene, the gene can still

be targeted by directing approved drugs in single, double and triple combinations to interacting genes.

Despite the relatively small number of FDA approved compounds, network approaches are a general, effective and accessible strategy for disease treatment (Kwong et al., 2012; Lin and Smith, 2011; Nijman and Friend, 2013; Pujol et al., 2010; Yang et al., 2010; Zou et al., 2013). Drug combinations also exhibit greater efficacy with fewer side effects and decreased toxicity compared to individual therapies (Sun et al., 2013).

In fact, the small world properties of biological networks may explain the common phenomenon in which unexpected therapeutic effects are obtained for drugs normally used in other diseases. For example, thalidomide was initially developed as a sedative but is now used to treat cancer (Sissung et al., 2009). Further, employing approved/developed drugs diminishes the need for preclinical testing. Many orphan diseases, in particular, have no available drugs (Sardana et al., 2011). Network guided therapy may provide options for these disorders.

Molecular networks can be used to guide drug combinations

The small world properties of biological networks have been used to design combination therapies for disorders including cancer, diabetes, neurodegenerative disorders and infectious disease (Csermely et al., 2013; Kwong et al., 2012; Nijman and Friend, 2013; Pujol et al., 2010; Yang et al., 2010; Zou et al., 2013). One study used highly time resolved transcript profiles and cell based phenotypes to show that EGFR inhibition reactivated apoptotic networks in breast cancer cells (Lee et al., 2012). These apoptotic pathways left the malignant cells susceptible to subsequent treatment with genotoxic drugs. Another investigation examined already employed therapeutic drug combinations

and merged these data with known drug-target interactions and protein–protein interactions (Zou et al., 2012). The integrated data could be used to successfully predict new drug combinations.

A different approach employed an algorithm that incorporated previously reported drug-drug interactions to predict new interactions (Guimera and Sales-Pardo, 2013). Stochastic block models that used the notion of group-dependent interactions were employed to infer networks in which the interaction between any drug pair was predicted by the group in which the pair resides. Another study increased the efficiency of discovery for drug pairs with synergistic interactions by combining pre-existing data from empirically determined interacting drugs with other data, such as protein interactions. The investigation used matrix algebraic technique based on cyclical projections onto convex sets (Gerlee et al., 2013).

Copy number alterations as a disease driver

For cancer, in particular, it is well established that amplification or deletion of genes plays a causative role. Amplification of the c-Myc gene and epidermal growth factor receptor (EGFR) genes, for example, have been strongly implicated in non-small cell lung cancer (NSCLC) (Sos et al., 2009) as well as a variety of other cancers (Beroukhim et al., 2010). Systematic surveys of DNA copy number alterations (CNAs) has linked cancer with a broad array of genes, both oncogenes and tumor suppressor genes. Further, the mechanisms by which the CNAs drive proliferation can be dissected using genomic techniques. For example, CNAs in glioblastoma have been connected to altered gene expression, which in turn has been related to survival (Jornsten et al., 2011). However, individual oncogenes have generally been studied in isolation. Co-

inheritance patterns for pairs of amplified and deleted genes, particularly those distant from each other in the genome, have been subjected to more limited scrutiny.

Using correlated copy number alterations to construct survival networks

Recent investigations have sought genetic interaction networks for cancer by seeking correlated patterns of unlinked CNAs. Genetic survival networks identified using correlated CNAs have been found in glioma cells (Bredel et al., 2009; Rapaport and Leslie, 2010) and ovarian cancer cells (Gorringe et al., 2010). Correlated patterns of CNAs in cancer that span entire chromosome arms have also been identified (Kim et al., 2013). However, the chromosome arm network highlights a problem of charting CNA interactions in cancer, namely amplifications and deletions are not distributed randomly over the genome. Rather CNAs are flanked by hot spots for DNA rearrangements and can incorporate many genes (Beroukhim et al., 2010; Hsiao et al., 2013). This poor resolution can make the identification of causative gene pairs difficult.

A pan-cancer CNA interaction network

In a relevant study, the resolution of identified CNA interactions was improved by combining data from over 4,000 different cancers across 11 different varieties (Zack et al., 2013). For each cancer type, there was a median of 74 consistent CNAs, summing to a total of 770 CNA regions over all varieties. Pan-cancer CNAs were identified by looking for alterations present in all cancers. The size of the significant CNAs decreased from 1.4 Mb in the individual cancers to 0.7 Mb in the pan-cancer CNAs, improving the resolution with which causative genes were mapped. Yet, by imposing the criterion that the CNAs were found in all cancers, the number of detectable events was diminished ~5-fold. Further, most pan-cancer CNAs still harbored more than one gene, often more than 200. It was possible to construct a network by looking for correlated CNAs in the

pan-cancer data. Not surprisingly, however, the size of the resulting network was small, with only 436 nodes.

Mapping genetic survival networks using correlated CNAs in radiation hybrid cells

Our group has used radiation hybrid (RH) panels to map genetic interactions critical for cell survival. Radiation hybrid (RH) mapping was invented to determine the relative locations of genes within mammalian genomes (Cox et al., 1994; Goss and Harris, 1975). RH panels are constructed by lethally irradiating cells, causing the DNA to fragment into small pieces. The irradiated cells are then fused to living hamster cells, which incorporate the DNA fragments into their genomes. The resulting hybrid cells each contain extra copies of a random assortment of genes (~25%), which are triploid rather than diploid. Genes in close proximity tend to be co-inherited in the RH clones, while genes far apart tend to be inherited independently. The small size of the DNA fragments affords the technique very high resolution, in fact, to within a single gene.

We showed that extra copies of distinct genes, unlinked triploid pairs, may enhance the survival of an RH cell (Lin et al., 2010). Because of the hardiness of the RH clones, statistically significant patterns of co-inherited genes pointed to the cell's survival mechanism. Over 7.2 million statistically significant interactions were identified using the RH data, including genes that partner specifically with oncogenes. The RH network was mapped at single gene resolution (<150 kb) (**Figure 1A**) and the fact that the network was Gaussian rather than scale-free indicated that nearly all of the network has been charted. In fact, the RH survival network overlaps significantly with other protein-protein interaction networks, while being hundreds of times more comprehensive.

A survival network for glioblastoma multiforme at single gene resolution

We explored the existence of survival networks in cancer (Lin and Smith, 2011). Correlated patterns of copy number alterations (CNAs) for distant genes in glioblastoma multiforme (GBM) brain tumors were identified using the same method employed to construct the RH survival network. We analyzed public data on 301 glioblastoma multiforme brain tumors, which had been assessed for CNAs using array comparative genomic hybridization (aCGH) with 227,605 markers (The Cancer Genome Atlas (TCGA) Research Network, 2008). The tumors had a mean amplification length of 5.35 Mb and a mean deletion length of 5.87 Mb. A total of 11.2% genes were amplified in more than 5% of the glioblastomas and 0.9% deleted. Copy number variations found in the normal population were excluded.

Pairs of amplified genes in the tumors were identified that were separated by more than the corresponding upper limit of the amplification lengths in the genome. Pairs of distant genes both of which were deleted were identified, or pairs of genes where one was amplified and the other deleted. We tested whether the amplification and/or deletion of the widely separated genes occurred simultaneously at a rate greater than by chance. A total of 436,302 interactions were found in the glioblastoma network at a false discovery rate (FDR) (Benjamini and Hochberg, 1995) < 5%. An example of a gene interaction between the Von Hippel-Lindau (VHL) tumor suppressor gene and the MAP/Microtubule Affinity-Regulating Kinase 2 (MARK2) gene is shown in **Figure 1B**. Unlike the RH interaction peaks, the GBM interaction peaks have multiple plateaus, representing non-random breakpoints in the tumor DNA. This phenomenon decreases mapping resolution for interacting genes.

The glioblastoma and RH survival networks overlapped significantly ($P = 3.7 \times 10^{-31}$, one-sided Fisher's exact test), validating the cancer network. We therefore exploited the high-resolution mapping of the RH data to obtain single gene specificity in the glioblastoma network. We identified overlapping interactions in the two networks to construct a cancer network featuring 5,439 genes and 13,846 interactions (FDR < 5%). This network suggested novel approaches to the therapy of glioblastoma. An example featuring the epidermal growth factor receptor (EGFR) oncogene is discussed below.

Using CNA networks to guide combination therapies

CNA networks represent a new opportunity to design combination therapies based on direct genetic interactions rather than proxy measures of interaction such as correlated gene expression levels. We focus on the single gene resolution CNA networks deduced from the RH and glioblastoma datasets. The principal therapeutic opportunity using these networks is for disorders of cell proliferation including cancer, autoimmunity and atherosclerosis.

In the following sections, we illustrate three strategies by which CNA interaction networks can be used to design network guided combinatorial therapies; (**1**) Using subnetworks to identify multiple drug targets that interact with a disease gene (**Figure 2**); (**2**) Using drugs to target multiple genes in a disease pathway (**Figure 3**); and (**3**) Using drugs to target genes in parallel pathways converging on a disease process (**Figure 4**). Drug/gene interactions In the examples were obtained from a number of databases, including DrugBank (http://www.drugbank.ca)(Knox et al., 2011) the Drug Gene Interaction Database (DGIdb; http://dgidb.genome.wustl.edu) (Griffith et al., 2013), GeneCards (Safran et al., 2010) (www.genecards.org), the Pharmacogenomics Knowledge Database (PharmGKB, http://www.pharmgkb.org) (Whirl-Carrillo et al., 2012)

and the Therapeutics Targets Database (http://bidd.nus.edu.sg/group/ttd/ttd.asp) (Zhu et al., 2012). Other databases can also be employed (Csermely et al., 2013; Sun et al., 2013; Zou et al., 2012).

Targeting multiple drugs to single disease genes in cancer

The c-Myc oncogene plays a major role in a wide variety of cancers (Wang et al., 2011). No approved compounds are available that specifically inhibit c-Myc, but a strategy that targets genes interacting with this gene product may be fruitful (Yang et al., 2010). In the RH survival network, 45 genes were linked with statistical significance (false discovery rate, FDR < 10^{-4}) to c-Myc. Of the genes that interacted with c-Myc, 12 (27%) happened to be specific targets for already existing drugs, though not necessarily for cancer treatment (**Figure 2A**). For example, the BMI1 polycomb ring finger oncogene product (PCGF4) is a subunit of an E3 ubiquitin ligase and is inhibited by the compound PRT4165 (Alchanati et al., 2009). Similarly, MAP2K5 (MEK5/ERK5) is a dual specificity protein kinase belonging to the MAP kinase kinase family and is inhibited by the compounds BIX02188 and BIX02189 (Tatake et al., 2008).

The epidermal growth factor receptor (EGFR) oncogene is frequently activated in glioblastoma and other cancers. Medications that target the EGFR oncogene include the monoclonal antibody cetuximab (Erbitux) and the kinase inhibitors erlotinib (Tarceva) and gefitinib (Iressa) (Stinchcombe et al., 2010). Eventually, however, resistance to these treatments occurs (Dhomen et al., 2012).

A total of 46 genes were identified that interacted with EGFR in the combined glioblastoma/RH survival network (FDR < 0.05), of which 10 (22%) happened to be targets for existing drugs (**Figure 2B**). For example, butyrylcholinesterase (BCHE) is

inhibited by donepezil, an anticholinesterase employed in treatment of Alzheimer's disease (Anand and Singh, 2013). SLC2A9 is a high capacity urate transporter and is inhibited by the uricosuric agent benzbromarone which is used to treat gout (Caulfield et al., 2008; Doring et al., 2008; Vitart et al., 2008). These observations suggest that a flank attack strategy which strikes at both EGFR and its partner genes in the glioblastoma survival network may be an effective approach for treatment of these tumors.

Patient-to-patient variations exist in disease networks. For instance, a variety of oncogenes are activated in different cancers (The Cancer Genome Atlas (TCGA) Research Network, 2008; Zack et al., 2013). Our strategy of using correlated CNAs to guide combination therapies can account for individual variations in disease networks, providing a foundation for personalized medicine.

Targeting multiple drugs to a single disease gene in autoimmunity

We have also used correlated CNA networks to design combination treatments centered on NFATc1. This gene plays a key role in T cell activation, an important cellular response in autoimmune disorders (Bartelt et al., 2009; Kannan et al., 2012; Smith-Garvin et al., 2009). In the RH survival network, 56 genes were linked with statistical significance (FDR < 10^{-4}) to NFATc1 (**Figure 2C**). No approved compounds exist that specifically target NFATc1. However, of the genes that interact with NFATc1, 9 (16%) happen to be specific targets for already existing drugs. One unsurprising example is PTGS1 (cyclooxygenase 1). This enzyme is involved in prostaglandin synthesis and is a target for non-steroidal inflammatory drugs (NSAIDs) (Dinarello, 2010). Another plausible example is the MECOM oncoprotein, which is specifically degraded by arsenic trioxide (ATO), perhaps explaining the promise of this compound in the treatment of autoimmune syndromes (Bobe et al., 2006; Shackelford et al., 2006). Other interacting

genes and their cognate pharmaceuticals were more unexpected and have yet to be used to treat autoimmune conditions. The enzyme SMPD4 (sphingomyelin phosphodiesterase 4) is inhibited by the compound GW4869 (Chipuk et al., 2012). Similarly, the TRIO gene encodes a rho guanine nucleotide exchange factor, which is specifically inhibited by the compound ITX3 (Bouquier et al., 2009).

Targeting multiple genes in a single pathway for cancer

The second strategy to design drug combinations with CNA network information targets gene products that participate in a single pathogenic pathway. An example of one such pathway in the RH survival network ends on the EGFR oncogene (**Figure 3**). (Note that this subnetwork does not incorporate information from the glioblastoma CNA network and may be a more general network than shown in **Figure 2B**.) A total of 22 genes interacted with the EGFR gene in the RH network (FDR < 10^{-6}), of which seven (32%) happened to be targets for existing compounds (**Figure 3**). For example, R59022 inhibits diacylglycerol kinase β (DGKB) (Batista et al., 2005; Kamio et al., 2010) and RGB-286147 inhibits PFTAIRE protein kinase 1 (PFTK1) (Caligiuri et al., 2005).

A trio of gene products that interacted with EGFR had antioxidant activity (**Figure 3**). Thioredoxin reductase (TXN) is inhibited by the gold compound, auranofin (Cox et al., 2008; Liu et al., 2012), which is also employed to treat autoimmune conditions such as rheumatoid arthritis. Peroxiredoxin 3 (PRDX3) is inhibited by thiostrepton, a thiazole antibiotic that shows activity against tumor cells (Newick et al., 2012). Glutathione-S-transferase (GSTT) is inhibited by α tocopherol, a form of vitamin E (Van Haaften et al., 2001), as well as by ellagic acid and curcumin, plant polyphenolic compounds (Hayeshi et al., 2007). There has been rising interest in inhibiting reduction/oxidation pathways for

cancer treatment, since these pathways are required for cell proliferation (Kwok et al., 2008; Newick et al., 2012; Tew and Townsend, 2011). One mechanism by which these pathways might exert their therapeutic effects may be exemplified by interactions with oncogenes such as EGFR.

The transforming growth factor β receptor 1 gene (TGFBR1) also interacted with EGFR (**Figure 3**). TGFBR1 is a target for a number of kinase inhibitors, including SB525334 and SD-208 (Akhurst, 2006; Mohammad et al., 2011; Thomas et al., 2009). A total of 72 genes interacted with TGFBR1 (FDR < 10^{-5}), of which 9 (13%) represented targets for available drugs. One of these genes was cysteinyl leukotriene receptor 2 (CYSLTR2), which is inhibited by available leukotriene inhibitors such as zafirlukast and zileuton. These compounds are used clinically as anti-inflammatory agents (Scow et al., 2007). Another gene that interacted with TGFBR1 was tachykinin receptor 2 (TACR2). Antagonists of this receptor include ibodutant and saredutant (Santicioli et al., 2013).

TGFBR1 also interacted with cytidine deaminase (CDA), which in turn interacted with matrix metalloproteinase-16 (MMP16) (FDR < 10^{-4}). CDA is inhibited by chemotherapeutic drugs such as tetrahydrouridine (Beumer et al., 2008) and zebularine (Lemaire et al., 2009). MMP16 is inhibited by marimastat (Wong et al., 2013). Both CDA (interactors FDR < 10^{-4}) and MMP16 (interactors FDR < 10^{-5}) were linked with a number of additional genes whose products can be antagonized by available drugs (**Figure 3**). The wide variety of existing drugs that target the EGFR pathway suggest that combinations of these compounds might have therapeutic benefits in applications in which this oncogene is a key node driving proliferation.

Targeting genes in parallel pathways converging on atherosclerosis

The third strategy that employs CNA networks to design combination therapies exploits parallel disease pathways. An example of this approach targets the multiple pathways that have been implicated in atherogenesis (**Figure 4**) (Lusis, 2012; Lusis et al., 2004). Apolipoprotein B (APOB) is the major protein constituent of low density lipoprotein (LDL) and elevated LDL concentrations are associated with increased atherosclerotic risk. Lipoprotein (a) (LPA) is a lipoprotein that also raises the risk of atherosclerosis through unknown mechanisms. The zinc fingers and homeoboxes 2 gene (ZHX2) and the Ox40 ligand (TNFSF4) have been implicated in atherosclerosis through genetic studies in mice and humans. There are no available drugs that directly affect any of these proteins. However, each of these atherogenic genes interact with between 5 to 9 genes (FDR < 10^{-4}) that are affected by existing compounds. Some of these drugs are already employed as anti-atherogenic agents.

For example, ZHX2 interacts with the prostaglandin E receptor 1 gene (PTGER1). Non-steroidal anti-inflammatory drugs (NSAIDs), such as aspirin and naproxen, inhibit the synthesis of the prostaglandin ligands for this receptor. These drugs are also widely used as prophylactic drugs to protect against atherosclerosis. The APOB gene interacts with the tyrosine kinases c-Kit (KIT) and MAPK14. KIT can be inhibited by kinase inhibitors such as imatinib and dasatinib (Ashman and Griffith, 2013). Similarly, phosphorylation of MAPK14 can be blocked using the kinase inhibitor sorafinib (Chapuy et al., 2011). The inference that kinase inhibitors may be beneficial in atherosclerosis is supported by recent studies (Grimminger et al., 2010; Hilgendorf et al., 2011).

The network connections of drug targets may explain their unexpected therapeutic effects in atherosclerosis. The angiotensin II receptor, type 1 (AGTR1) is significantly

linked to APOB in the RH network (**Figure 4**). The angiotensin converting enzyme (ACE) inhibitors (e.g. enalapril), and the angiotensin receptor blockers (ARBs) (e.g. losartan) are effective in combating atherosclerosis (Patarroyo Aponte and Francis, 2012). The connection of AGRT1 with APOB might explain part of the efficacy of angiotensin pathway blocking agents as anti-atherosclerotic drugs, in addition to their role as antihypertensive agents.

Using CNA networks to synergize drug combinations and minimize side effects

Network guided combination therapies might allow the use of multiple high efficacy drugs at low concentrations or alternatively, combinations of low efficacy drugs. One potential example of this synergistic strategy is provided by marimastat, which inhibits MMP16 in the RH pathway terminating on EGFR (**Figure 3**). Marimastat is not used clinically because of an unacceptable side effect profile (Wong et al., 2013). By combining marimastat at low concentrations with other drugs in a network guided strategy, it might be possible to maximize their common therapeutic effects, while minimizing the divergent adverse effects. Nevertheless, accumulating side-effects will eventually set limits to polypharmacy. The optimal balance between therapeutic synergism and gathering side-effects will require empirical investigation.

Based on network data alone, it is not always possible to predict the direction of a drug effect. For example, APOB interacts with histone deacetylase 7A (HDAC7A) (**Figure 4**). HDAC7A is a class II HDAC, and is a target for inhibition by histone deacetylation inhibitors (HDIs). In fact, recent studies indicate that HDIs show promise in the therapy of atherosclerosis (Ordovas and Smith, 2010; Xu et al., 2012; Zhou et al., 2011). However, the HDI trichostatin A targets HDAC7A, but is proatherogenic in mouse models (Choi et al., 2005), underlining the necessity of experimental testing.

Nevertheless, the strategy of CNA network guided combinatorial therapy promises to be a useful approach to advancing novel treatments for a wide variety of common and uncommon disorders.

Conflict of interest

The authors declare no conflict of interest.

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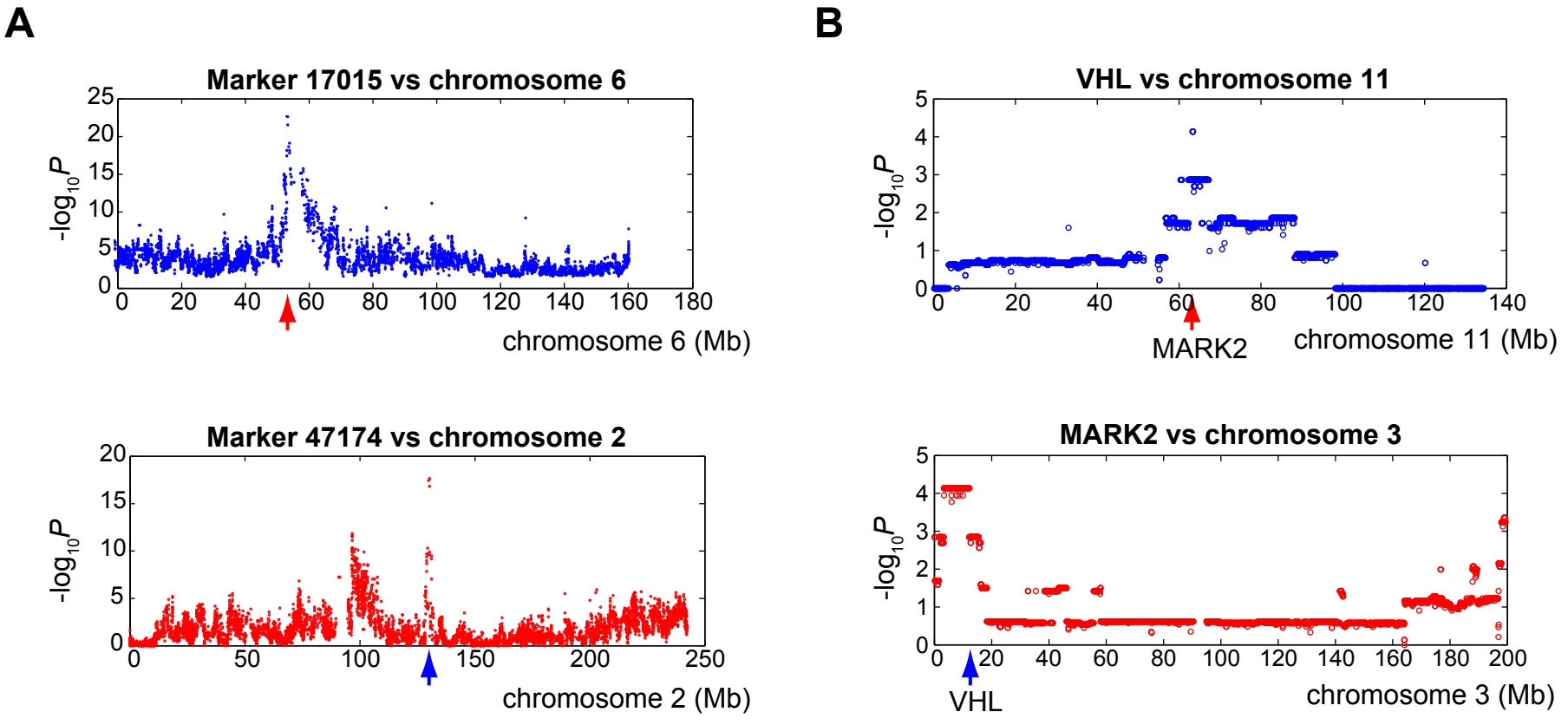
FIGURE LEGENDS

Figure 1. Genetic interactions in RH and GBM cells. (**A**) An interaction between a gene on chromosome 6 (red arrow) and a gene on chromosome 2 (blue arrow) in the RH network (Lin et al., 2010). The ordinate shows the significance value ($-\log_{10}P$) for co-retention. (**B**) An interaction between the MARK2 gene on chromosome 11 (red arrow) and the VHL gene on chromosome 3 (blue arrow) in the glioblastoma network.

Figure 2. Using subnetworks to target individual node genes. (**A**) Subnetwork for c-Myc and all genes one edge away in the RH survival network (FDR < 10^{-4}). Genes in red are targets for existing drugs. (**B**) A subnetwork for the EGFR gene in the combined RH/glioblastoma network (FDR < 0.05). (**C**) Subnetwork for the T cell activation gene NFATc1 and all genes one edge away in the RH network (FDR < 10^{-4}).

Figure 3. Targeting an individual pathway. A pathway leading to the EGFR oncogene in the RH network. Genes in red are targets for existing drugs. Genes that are non-drug targets are not shown. (FDRs for interacting genes: MMP16 < 10^{-5}, CDA < 10^{-4}, TGFB1 < 10^{-5}, EGFR < 10^{-6}.)

Figure 4. Targeting parallel pathways. Genes that conspire to promote atherogenesis in the RH network. Genes in red are targets for existing drugs. Except for the node genes APOB, ZHX2 and LPA, non-drug targets are not shown. (FDRs for interacting genes < 10^{-4}).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chromosome 3 (Mb)

Figure 1

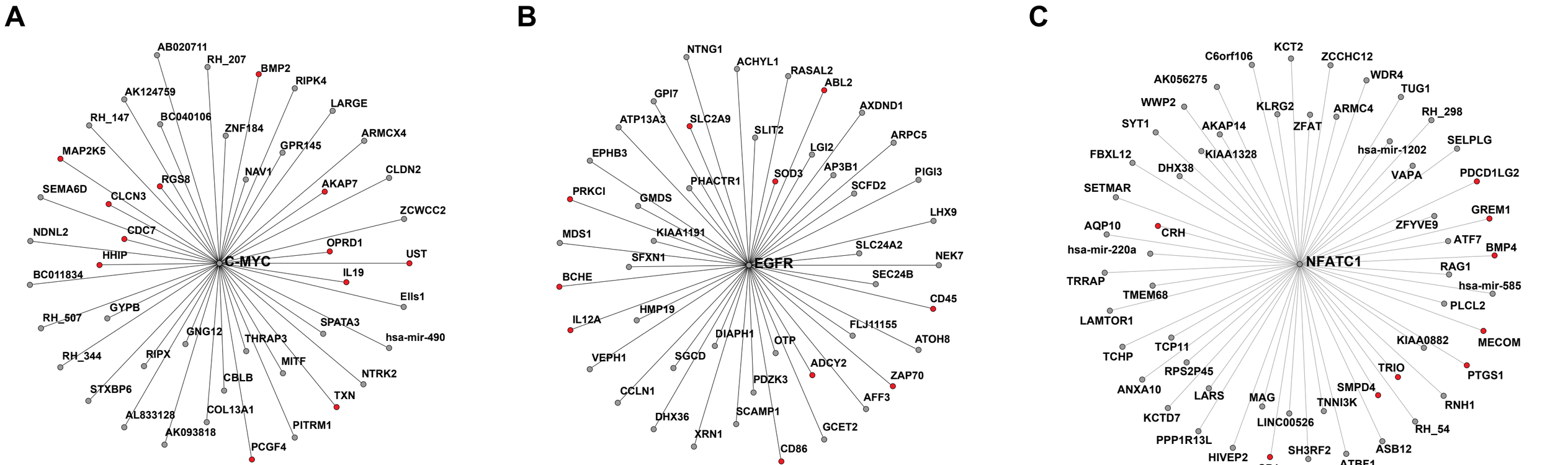
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Figure 2

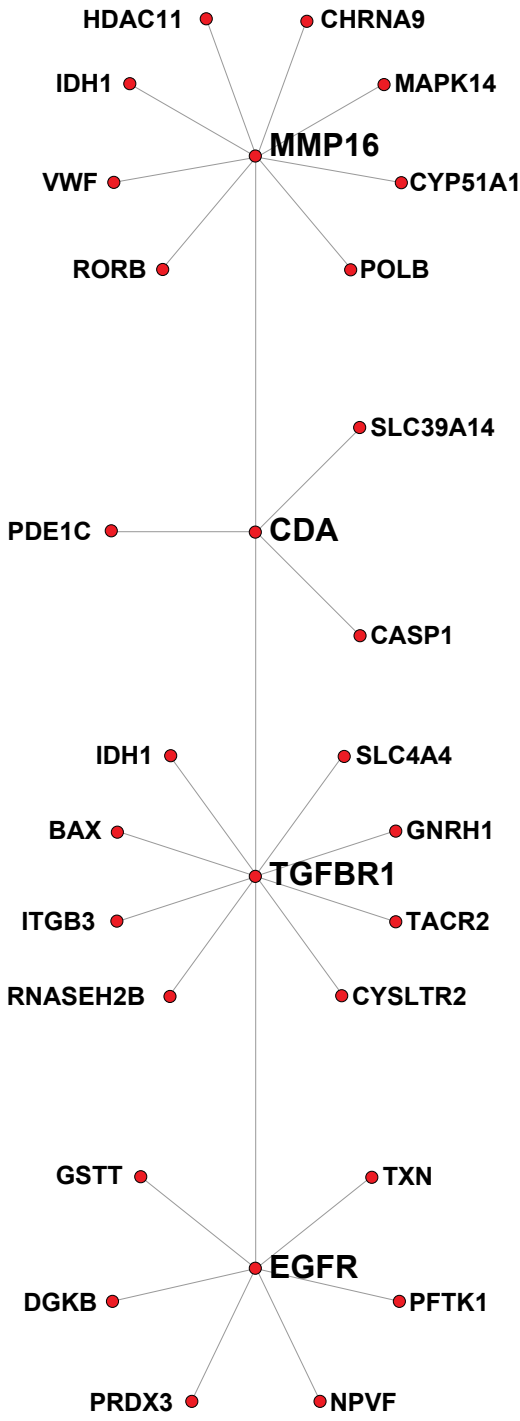
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Figure 3

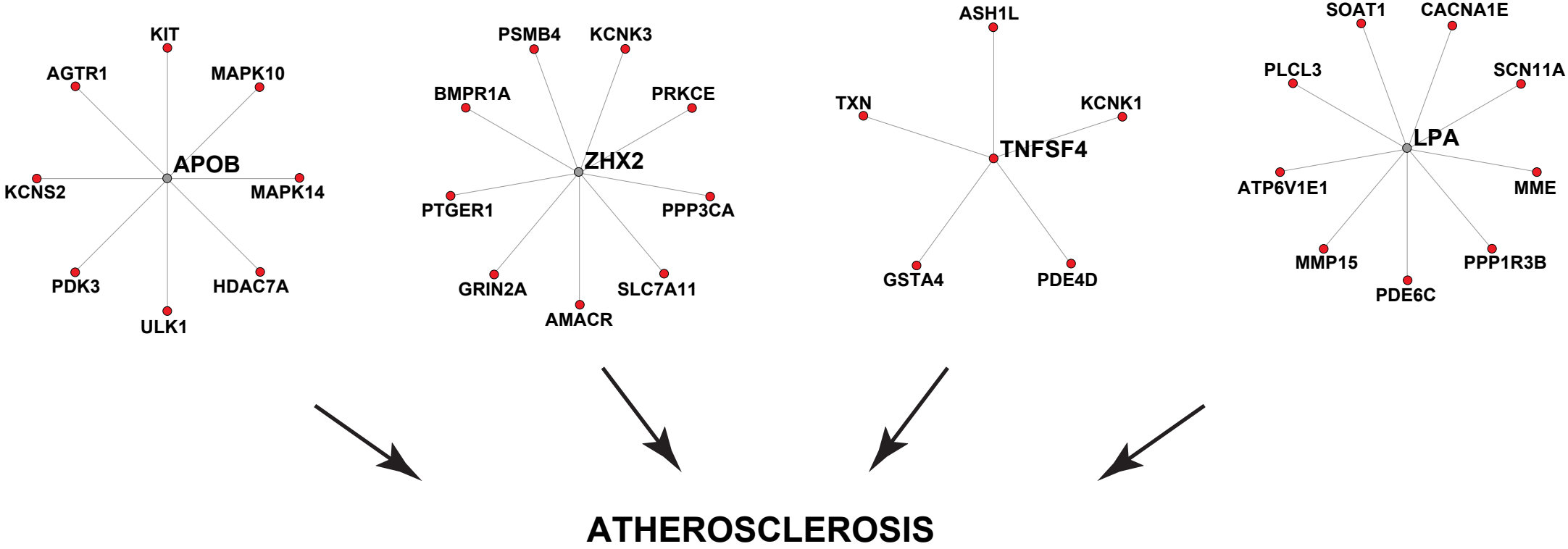
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Figure 4