Collateral Lethality: A new therapeutic strategy in oncology

KM plotter

Genomic deletion of tumor suppressor genes (TSG) is a rite of passage for virtually all human
cancers.
In the course of genomic events that delete TSGs, a large number of genes with no apparent direct
role in tumor promotion also sustain deletion as a result of chromosomal proximity to the target
TSG.
Collateral lethality – deletion of cancer specific vulnerability due to co-deletion
Genetic changes - recurrent activating events (point mutations, aberrant fusions, and amplifications of proto-oncogenes) as well as loss-of-function events (point mutations, genomic deletions, and
aberrant fusions of tumor suppressor genes (TSGs))
Synthetic lethality - interaction between two genes in which inactivation of either of them is compatible with life but co-inactivation in the same cell or organism leads to death
-so can inactivate a gene in cancer cells to cause cell death which does not affect the normal cells

the interaction between the tumor suppressors and their partners was not "**synthetic lethal"** but rather "**synthetic sick**", meaning that the co-occurrence of events leads to a growth impairment that is still compatible with life
Synthetic lethality - TP53, PTEN, and CDKN2A
Many genes do not have house keeping functions
Example:
-BRCA1/2 (BReast CAncer gene) loss-of-function mutations or deletions with inhibitors of PARP (poly(ADP-ribose) polymerase,)
-BRCA sensitizes cancer cells to PARP inhibitors because both of these genes play redundant roles in a specific DNA double strand break repair
-knockout of PARP1 in the context of mutant BRCA1 is lethal, while both knockouts are viable on their own
- the above-mentioned tumor suppressor genes, compromises DNA repair, a major housekeeping function

Genomic deletions and "passenger" genes
-occur stochastically and clonally expand if they confer a biological advantage to the aspiring cancer cell
-example: Loss of one arm of chromosome with wild type genes when the other chromosome has loss of function mutation -Knudson's two-hit hypothesis
loss of heterozygosity" (LOH), whereby loci that are heterozygous in somatic tissues become hemizygous in cancer due to loss of one allele by genomic deletion
eg: RB1, WT1, NF1, APC and PTEN
-**Signature events:** Different tumor types exhibit highly recurrent and specific heterozygous deletions

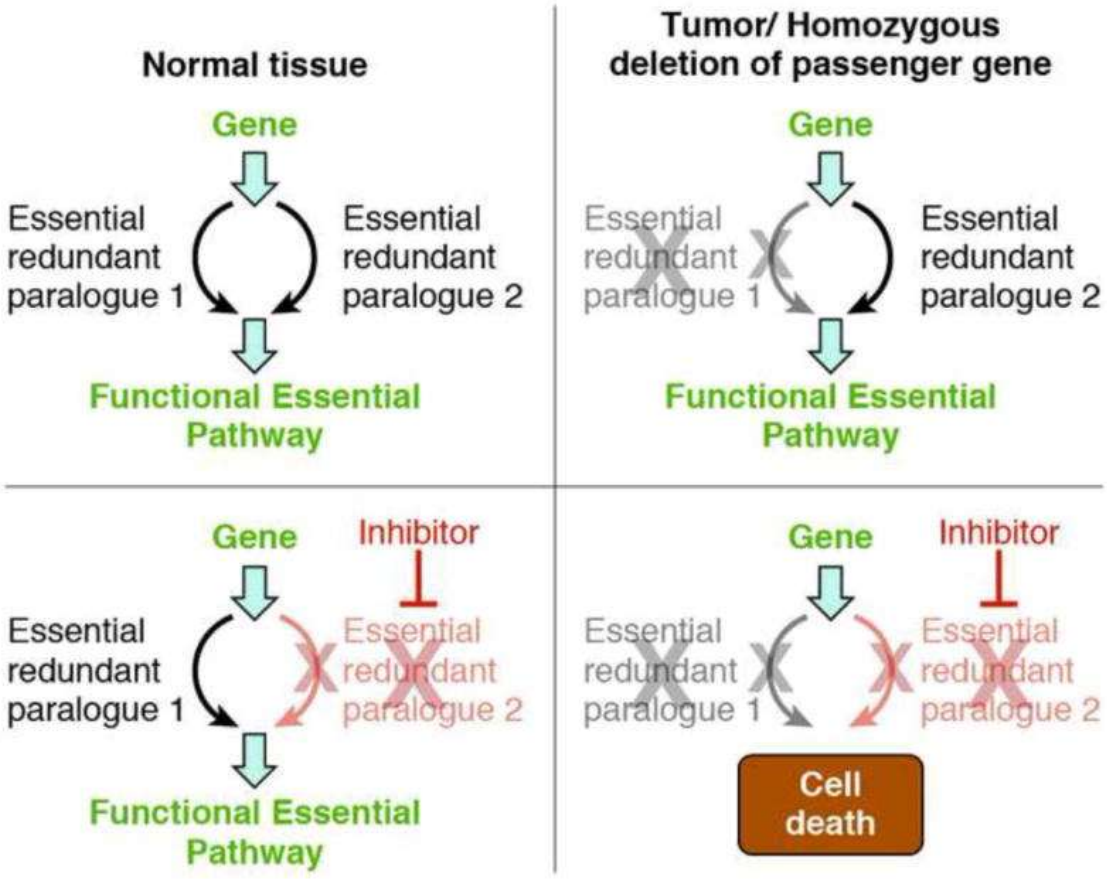
While **heterozygous deletions are ubiquitous** in human cancer, homozygous deletions are more restricted and their frequency varies between cancer types
high load of homozygous deletions) also trend towards a worse prognosis

Contrary to drivers, passenger deletions - little or no biological and therapeutic significance. However
But many **passenger deleted genes** play important roles in diverse metabolic and housekeeping functions, yet cell viability is maintained due to **functional redundancy** of related gene families
Examples:
1p36 locus (Figure 1), which targets multiple tumor suppressor genes but can also include ENO1 (glycolysis), NMNAT1 (NAD+ biosynthesis), and PGD (pentose phosphate shunt).

Collateral lethality: Synthetic Lethal Targeting of Passenger Deleted Genes

1. **Targeting redundant paralogues of gene families performing cell essential functions**

Passenger deletion of critical metabolic enzymes appear to be tolerated by cancer cells due to the co-expression of partially redundant, closely related paralogues which serve to maintain these essential cellular metabolic reactions
-dependent on the paralogue and deleting or inhibiting will lead to cancer cell lethality



Glioblastoma:
-glycolytic gene ENO1, which is homozygously deleted as part of the 1p36 tumor suppressor locus
-Enolase is a key enzyme for cellular bioenergetics, without which glycolysis cannot produce ATP.
-3 paralogous - ncoded by three redundant homologues with glioma cells only expressing ENO1 and ENO2, the latter gene is located on chromosome 12
-glioma cells with deletion of ENO1 are exceptionally sensitive to the ablation of ENO2, by both genetic (shRNA) and pharmacologic means
-other genes metabolic passenger deleted genes NMNAT1, PANK1 and ACO1

Lung cancer cells: loss-of-function mutations/deletions of the chromatin remodeling helicase SMARCA4 sensitizes lung cancer cells to ablation of its paralogue, SMARCA2
BUT **SMARCA4** is a **true tumor suppressor** and its inactivation is a driver rather than passenger event. That said, unlike most tumor suppressors, SMARCA2/4 exert a genuine cellular housekeeping function rather than merely restricting cellular growth

Genetic screen -homozygously deleted genes – genetically essential/house keeping genes- CRISPR editing

2. **Targeting passenger deleted genes based on non-homologue based genetic/biochemical redundancy**

- many passenger deleted genes could cause *biochemical alterations that render otherwise non-essential biochemical pathways essential* even if they are not members of an essential/redundant gene pair
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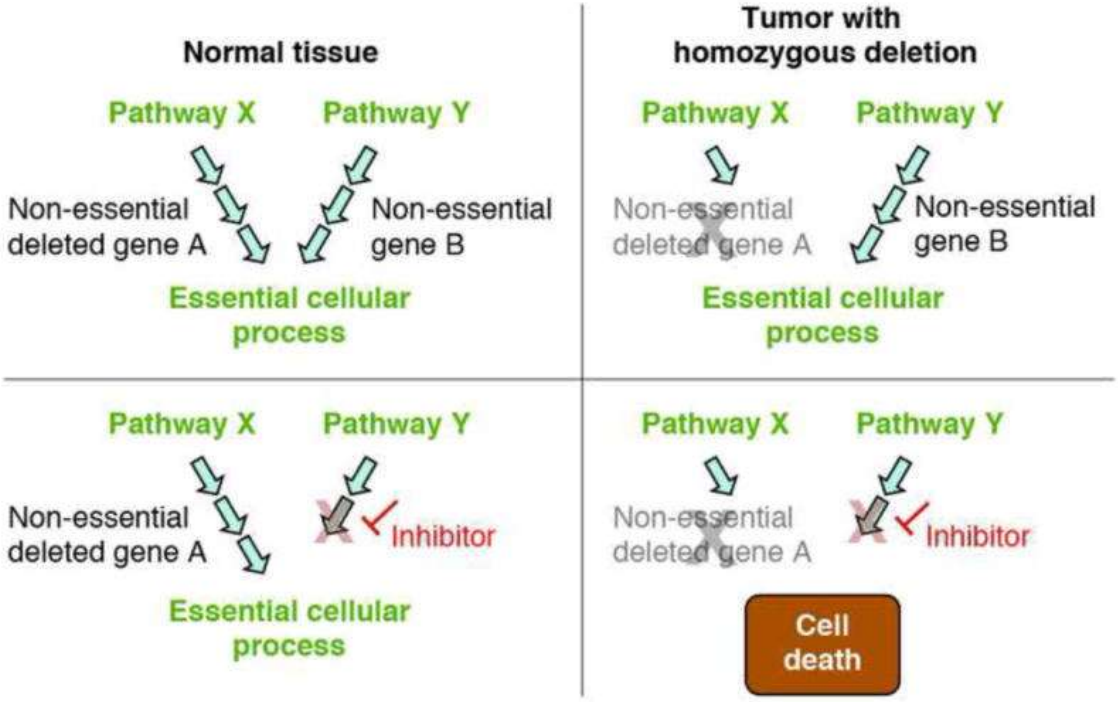
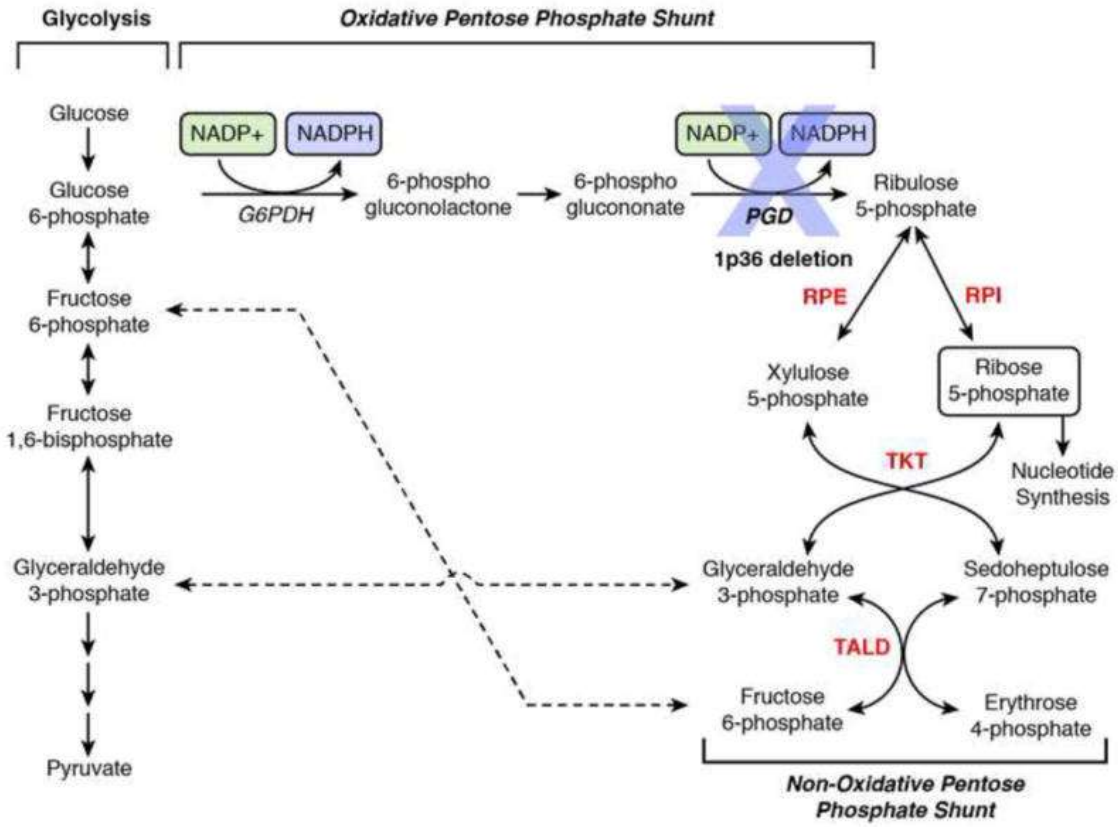


Two (or greater) biochemical pathways can lead to the same essential cellular process. Passenger homozygous deletions can affect one of those pathways while leaving the other

intact, thus having no detrimental effect on cancer cell viability but causing the remaining pathway to become essential.

deletion of 6-phosphoglucinate dehydrogenase (PGD) on the 1p36 locus as an illustrative example. PGD is a key enzyme of the oxidative pentose-phosphate shunt, but is not a cell-essential gene per se because NADP+ can be produced from other sources and ribose-5-phosphate, which is essential for nucleotide synthesis, can still be produced from the non-oxidative pentose phosphate shunt



Nucleotide biosynthesis for DNA and RNA requires Ribose-5-phosphate, which can be derived from either Glucose-6-phosphate through the oxidative arm of the pentose phosphate shunt or the glycolytic intermediates, fructose-6-phosphate and glyceraldehyde-3-phosphate, through the non-oxidative arm.

combined loss of 6-phosphogluconate dehydrogenase (PGD) with either transketolase (TKT), transaldolase (TALD) or Ribulose Isomerase/Epimerase (RPI/ RPE)

A large body of work has been dedicated to exploiting biochemical vulnerabilities exposed by deletion of methylthioadenosine phosphorylase (MTAP). MTAP is immediately adjacent to CDKN2A and is one of the most frequently homozygously deleted genes in human cancer. Whether MTAP is a genuine tumor suppressor gene remains an area of active investigation.

While the full biochemical consequences of MTAP deletion remain to be elucidated, there is general agreement that it plays a role in salvage of methionine and adenosine.
Attempts have been made to exploit these vulnerabilities, in particular using inhibitors of purine biosynthesis.
While this area has great potential, these attempts have yet to bear fruit, possibly due to the **high extracellular concentrations of MTAP produced by stromal cells which may serve as a reservoir**

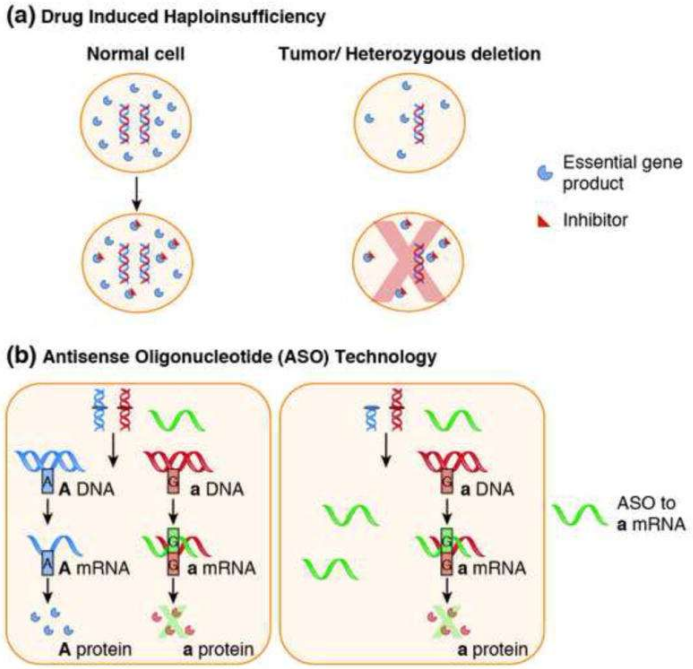
Collateral lethality targeting of heterozygously deleted genes
Drug-induced haplolethality

- Heterozygous deletions occur much more frequently in cancers than homozygous deletions.
- For instance, while the 1p36 homozygous deletion is found in around 2.5% of glioblastomas cases (typically cover an average of ten genes),
- -the heterozygous deletion is found in 25% of glioblastomas and around 15% of all cancers (covering hundreds of genes

Eg: ENO1 in chromosome 1
drug-induced haploinsufficiency" or "drug-induced haplolethality

Allele-specific targeting
Genomic variation between different alleles of the same gene can be another way to exploit specific vulnerabilities provided by heterozygous deletions via an approach called "variagenic targeting

selectively targeting tumors with loss of heterozygosity by allele-specific, oligonucleotide based knock-down of essential genes that are heterozygous in somatic tissues but become hemizygous by virtue of deletion in cancer



Genetic variation occurs in about 1 in 300 nucleotides in the human genome [75]. This variation is present in different alleles of the same gene, causing alterations that have neither significant phenotypic nor functional effects. Antisense oligonucleotide (ASO) technology can be used to target essential genes that are heterozygously deleted in cancers. These oligonucleotides are able to target polymorphisms that only differ by as little as one pair from each other, thereby inactivating one allele while leaving the other intact. In the figure above, an essential gene is encoded by alleles A and a. Tumor cells contain a heterozygous deletion in allele A. By introducing an antisense-oligonucleotide (ASO) directed against allele a (green), it is possible to cause selective death in cancer cells while leaving normal tissues intact.

3. Tumor heterogeneity and the effectiveness of collateral lethality target

for collateral lethality to be 100% effective, all tumor cells must carry the genetic event being targeted, otherwise a non-deleted resistance clone will likely grow out

- multiple modalities targeting different genetic events will ultimately have to be combined to achieve true tumor elimination like in HICV therapy
- the deletion is “clonal” rather than “sub- clonal