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1 **Evolutionary dependencies show the paths to cancer development**

2
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7 8 9 **Patterns of co-occurring and mutually-exclusive mutations reveal synergistic interactions** 10 **between cancer driver genes. A new study functionally confirms these interactions and builds** 11 **the pairwise relationships into networks of pathway disruption that have better predictive** 12 **power than relying on specific mutations.**

13
14 Cancers are clonally expanding populations of cells in which the normal regulation of cell division
15 and behaviour is undermined. That dysregulation is inherited by daughter cells and is thought to be
16 facilitated by a small number of driver mutations. Genome sequencing studies comparing samples of
17 cancer and normal cells from the same patient have been hugely successful at identifying the most
18 commonly occurring driver mutations¹. While it appears that most cancers have between one and ten
19 principal drivers², it is also apparent that these mutations don't simply work in an additive manner to
20 transform normal cells into cancer. Writing in this issue, Mina et al³ set out to develop the idea that
21 some driver mutations cooperate and so tend to co-occur in cancers, whereas others would be
22 functionally redundant and tend to occur mutually-exclusively.

23
24 Studying the patterns of mutation co-occurrence and mutual-exclusivity are collectively referred to as
25 evolutionary dependency (ED) analysis⁴⁻⁸. This new work substantially develops the concept beyond
26 just pairwise dependencies, it systematically tests both assumptions of and predictions from ED, and
27 gives a taste of the future insights that may be obtained from this style of analysis.

28 29 **Functionally validated dependencies**

30 Standing on the shoulders of The Cancer Genome Atlas (TCGA)¹, Mina et al³ curated a list of
31 recurrently mutated genes and their putative driver mutations. With a broad brush these were
32 annotated as either oncogenes that drive cancer by dysregulated activity or gain of function, or tumour
33 suppressor genes, where loss of activity enables cancer growth.

34
35 In an important validation of these driver annotations and proof-of-principal for later analyses, the
36 authors turned to publicly available data from high-throughput gene essentiality screens of cancer
37 derived cell-lines. These screens measure the relative fitness of cells - based on their ability to divide -
38 after using short hairpin RNAs or CRISPR editing to deplete the products of a target gene⁹⁻¹².

39 Knowing the driver mutations already present within each cell-line, the authors asked if, as expected,
40 depleting an oncogene with an activating driver mutation reduces cell fitness more than depleting the
41 same gene in a cell-line that does not have the driver mutation. This worked remarkably well,
42 confirming that the oncogene driver mutations were an important component of cell-line fitness, and
43 to a lesser extent an equivalent strategy also validated tumour suppressor genes.

44
45 Having functionally validated many of the driver mutations they scored all pairwise combinations of
46 drivers for co-occurrence and mutual-exclusion in the TCGA samples, finding a compelling excess of
47 both patterns - suggesting a wealth of ED relationships.

48

49 A key insight was that the gene essentiality screens could be used to test these evolutionary
50 dependencies and probe the nature of their interactions. For a pair of genes with an ED relationship,
51 they identified cell-lines that contained driver mutations in both genes (double mutants), either gene
52 (single mutants) or neither gene (wild-types), and asked how they responded to the depletion of one of
53 the genes in the pair. Depleting a gene from a pair with co-occurring mutations tended to produce a
54 much greater reduction in fitness for the double mutant cell-lines than for the single mutants (Fig. 1),
55 supporting the notion of synergy between co-occurring driver mutations.

56

57 For pairs of driver mutations that tend to occur mutually-exclusively in cancers, depleting the mutated
58 gene in single mutant cell-lines was found to be highly detrimental, more so than in double-mutant
59 cell-lines. This supports the expectation that mutually-exclusive mutations are often redundant
60 perturbations of the same cellular pathway.

61

62 Genetics has taught us to treasure our exceptions¹³. For the functional validation of co-occurring
63 drivers there was a single prominent outlier, whereas ED predicted synergy between KRAS and
64 STK11, the essentiality assays consistently point in the opposite direction, suggesting that STK11
65 mutations reduce a cell's need for KRAS driver mutations. Amongst mutually-exclusive driver pairs
66 the exceptions were common, approximately 30% of significant essentiality assays indicating driver
67 synergy, rather than ED implied redundancy. Reconciling these exceptions may provide greater
68 insights than the confirmatory results, as they likely reflect the influence of the tumour
69 microenvironment on driver gene interactions and could point to targetable cancer vulnerabilities.

70

71 **Pieces of larger puzzles**

72 Pairwise evolutionary dependencies are pieces of a larger puzzle. Mina et al³, used ED to build
73 “axes”, networks of co-dependency and mutual-exclusivity, the aim being to classify cancers by the
74 combinations of pathway perturbations that recurrently drive cancer development. Classification by
75 axis appears to be better at predicting prognosis and drug response than stratifying on a single key
76 driver mutation. Some of the drug responses in particular, such as the PARP-inhibitor sensitivity of
77 PIK3CA/NFE2L2 double-mutants, would not have been predicted nor readily detected without axis
78 based stratification.

79

80 The ED and functional validation approaches could be applied to other related puzzles such as
81 whether distinct driver mutations in the same gene are functionally equivalent and contribute to the
82 same axis. As cancer driver mutations appear to be common in non-cancer clonal expansions of cells
83 that are a typical feature of aging^{14,15}, a comparison of ED axes between cancers and non-cancer
84 clones may capture the distinctions between benign clonal expansion and cancerous transformation.
85 Evolutionary inspired insights have a bright future lighting the paths of cancer development.

86

87

88

89 **Fig. 1 | Paths of cancer development revealed by evolutionary dependency. a** Patterns of
90 significantly co-occurring or mutually-exclusive driver mutations (filled) identified for pairs of genes
91 from cancer cohorts. **b** Gene product depletion in cell-lines often confirms synergy between co-
92 occurring driver mutations and redundancy for mutually-exclusive mutations. Only one gene of the
93 ED pair is depleted (red border) and the relative fitness compared between cell-lines stratified by the
94 combined driver status (driver=filled, wild-type=open) of each gene. **c** Pairwise relationships can be

95 built into networks, within which clusters of co-occurring genes define an axis of cancer development.
96 **d** Classifying cancers by axis can have greater predictive power than using single driver genes.
97

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