Cell molecular signalling dynamics, including noise, are pre-tuned by cell phenotypic state

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MOTIVATION

Biological cells are far from identical, even within genetically uniform populations grown under identical conditions. Within a tumour, individual cells occupy distinct positions along continuous *phenotypic landscapes* — manifolds of morphological, molecular, and functional variation including the epithelial-to-mesenchymal (E/M) continuum and the cell cycle oscillator. Crucially, how the processing and transduction of external (molecular) signals is tuned by differences in these pre-existing cell phenotypic states remains largely unmapped [1, 2].

This is important because signalling variability — heterogeneous responses to identical stimuli across an isogenic cell population — is a major barrier to effective cancer therapy [3, 4]. While stochastic molecular noise is widely invoked as the cause of this variability, emerging research hints that some signalling variability may be *structured*: predetermined by the cell's current phenotypic state [5]. This raises three interlinked questions: (i) Do E/M state and cell cycle phase systematically organise the magnitude, timing, and spatial routing of signalling responses across diverse molecular networks? (ii) Is the degree of signalling variability itself state-dependent? (iii) What factors may control the 'noisiness' of molecular signalling, and how might this contribute to cell survival or selection. These questions relate to how complexity arises and whether it can be controlled [6].

APPROACH & METHODOLOGY

Two complementary single-cell imaging experiments were performed on lung cancer (A549) cells.

Experiment 1 (state mapping): Cells spanning epithelial-like and mesenchymal-like phenotypes were stimulated with five oncogenic ligands (EGF, TGF-β, IL-6, TNF-α, IFN-γ) across five timepoints, yielding >300,000 single-cell observations measured via 50-plex cyclic immunofluorescence (CycIF) [7]. A stimulation-invariant *cell-state manifold* was constructed by embedding only stimulus-unresponsive features (651 of 1,334) via PCA and UMAP, with cell states identified by graph-based clustering and cell cycle phase assigned via pseudotime inference. Signalling was analysed across five dimensions: response magnitude, kinetics, subcellular relocalisation, manifold alignment (neighbourhood-preservation between state and signalling landscapes), and variability (bootstrapped CV within vs. across states).

Experiment 2 (causal induction): To test whether cellular stress *causally induces* signalling heterogeneity, cells were treated with four pharmacological stress agents targeting distinct pathways: Thapsigargin (ER stress), Menadione (oxidative stress), Doxorubicin (DNA damage), and Docetaxel (mitotic stress). A 21-marker panel covering primary signalling, stress-state indicators, and cell-state features was acquired, followed by stimulation with EGF, IL-6, or IFN-γ. To classify the topology of stress-induced variability, a peak-guided distribution analysis was applied: kernel density estimation (KDE) of each response distribution, biologically-constrained peak finding, and Gaussian mixture model (GMM) fitting with the observed peak count k. Stable k indicated noise amplification; changing k indicated subpopulation emergence or loss.

KEY RESULTS

E/M state organises signalling magnitude, kinetics, and intracellular routing. Across all five stimuli, E/M state was the dominant determinant of signalling response. Signalling magnitude, kinetic trajectories, and subcellular relocalisation patterns all varied systematically between epithelial-like and mesenchymal-like states. E/M state also dictated subcellular routing, changing the intracellular contexts within which signals propagate to induce different outcomes. Surprisingly, cell cycle phase had little influence on signalling across these dimensions, implying that signalling responses are remarkably robust to changes across the proliferative cycle.

Stressed cell states selectively amplify signalling noise. A subset of cell states showed elevated within-state signalling CV relative to the total population — indicating that phenotypically similar cells in these states respond more variably, not more uniformly. This selective noise amplification was not distributed randomly across the state landscape: its frequency correlated

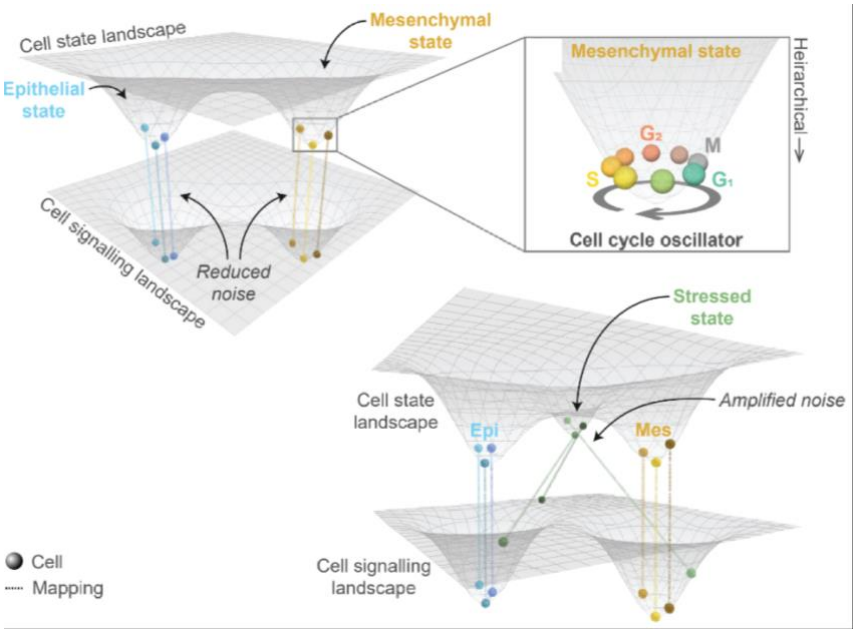
with the strength of a multi-molecular signature of cell stress assessed across cell state clusters. Stress was shown to be causal (not merely correlative) by pharmacological stress-induction (Thapsigargin, Menadione, Doxorubicin, Docetaxel), which consistently elevated signalling CV relative to time-matched controls.

The dominant topology is noise amplification; bifurcation occurs in a minority of cases. Distribution topology analysis of 120 stress–stimulation–marker combinations showed that most stress-induced CV increases (51%) reflected unimodal widening — a single population becoming noisier. However, in 7% of cases, pharmacological stress created a new discrete subpopulation peak, indicating genuine bifurcation into distinct signalling attractors rather than graded noise. A further 5% showed subpopulation loss. These data establish that while noise amplification is the predominant response to stress, a subset of stress–pathway combinations drives qualitative transitions in population structure.

CONCLUSIONS & OUTLOOK

These experiments and analyses establish that individual tumour cells function as *complex, heterogeneous information-processing units*. As depicted in the schematic figure, position on the cell state landscape largely pre-determines the way that incident stimuli are decoded on the cell signalling landscape; with E/M state being far more influential than cell cycle stage. Notably, the selective amplification of signalling noise in stressed cells may constitute a *bet-hedging strategy* — an 'every cell for itself' regime in which response optimisation shifts from the individual cell to the population as a whole, diversifying outcomes to maximise collective survival under adversity [8, 9]. Noise is therefore not merely a failure of precision but a potentially adaptive feature of tumour complexity. Future work will apply this framework to patient-derived clinical samples to connect single-cell signalling topology to treatment heterogeneity in lung cancer [10].

FIGURE



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Ethics Statement. All experiments used established human-derived cancer cell lines (A549). No human participants or animal subjects were involved.