CELL PLASTICITY & EPIGENETICS

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Phenotypic plasticity, the ability of a genotype to produce a variety of phenotypes, has been documented as a core biological process underlying numerous cellular events ranging from unicellular adaptation to multi-cellular organism development. In the context of cancer, phenotypic plasticity leads to the establishment of co-existing phenotypically diverse metastable states that could grant cancer cell populations with the capability to adapt to fast-paced environmental fluctuations in the absence of genetic divergence and thus fuel cancer development, metastatic spread and resistance to therapeutic paradigms. Given the crucial role that cell plasticity and non-genetically encoded phenotypes play in biology, our research aims to unravel the molecular mechanisms underlying such a phenomenon and to address its role as a key determinant during cancer onset and progression.

Over the past few years and by means of applying and developing multimodal single-cell technologies, our lab has demonstrated that clonal populations of a variety of cellular systems from diverse tissues of origin display multiple non-genetically encoded metastable states that can be ascribed to dramatically different cellular phenotypes. Motivated by our observations, and by applying our in-house developed lineage tracing technology (Figure 1A. BdLT-Seq (Shlyakthina, Blochl *et al.*, 2023, N*at Commun*; Shlyakthina, Blochl *et al.*, 2024, *STAR Protoc*)) we have recently shown that in *in vitro* transformation models driven by mutant HRAS, KRAS and NRAS, transcriptome states are inherited upon cell division but they can also be rewired into distinct states resulting in a progeny displaying diverging transcriptome profiles, thereby fuelling the generation of non-genetically heterogeneous populations. Moreover, we reported that the plastic capacity of transcriptome states to generate progeny with a transcriptome profile different from that of the parental cell is not stochastic, but it is rather encoded in non-genetic networks and restricted in an ancestry lineage-linked manner.

More recently, we have shown that restricted transcriptome plasticity and inheritance is not unique to cancer models and could be observed in non-tumourigenic cell systems suggesting its widespread and, perhaps, universal nature. Strikingly, and in line with BdLT-Seq data (Figure 1B), subcloning a parental – clonal – population of any of the cellular systems analysed gives rise to populations of cells that are enriched in subsets of transcriptome states (Figure 1C) that only partially recapitulate the heterogeneity observed in the parental clone, supporting the existence of a non-genetic molecular memory being transferred along cell divisions[3]. Strikingly, subclones enriched in distinct states show significant variations in their response to various environmental cues (e.g., anchorage-independent growth, anticancer drugs, oncogenic transformation) suggesting that metastable states may play a key role in shaping cancer onset, progression, and evolution.

Interestingly, by delving into the inner molecular workings underlying cell plasticity, we have uncovered that a large subset of long non-coding RNAs (lncRNAs) and a small fraction of intrinsically disordered proteins (Intrinsically Disordered Regions-containing proteins, IDRs) determine clonal molecular divergence, thus potentially acting as key players in cell plasticity and non-genetically supported cell and, thereby, populational adaptation (Shlyakthina, Blochl, Moran *et al.*, 2024, *bioRxiv*). Strikingly, we observed that divergent lncRNAs co-localize with cluster-specific IDR-proteins (Intrinsically Disordered Regions-containing proteins) within perinuclear structures and nucleate active components of major signalling pathways

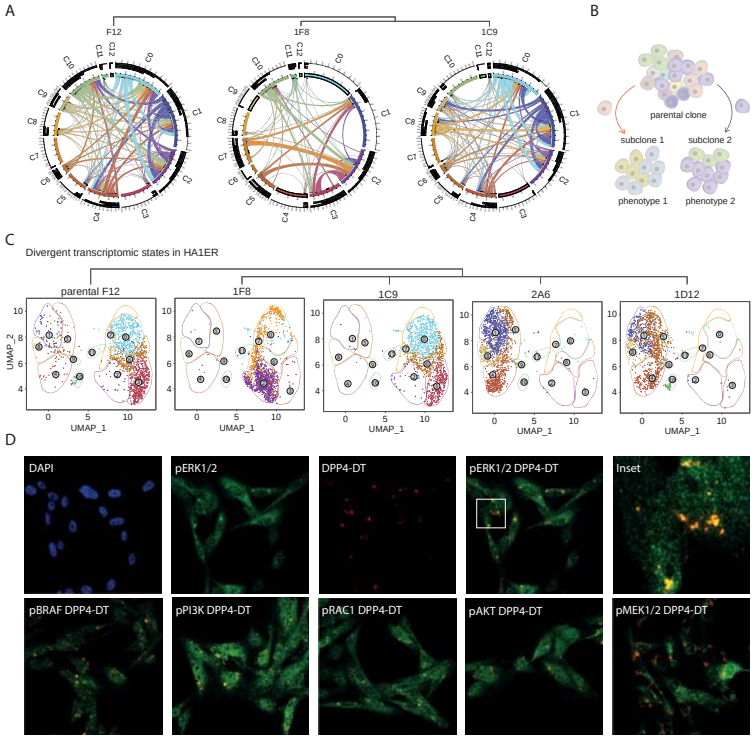

Figure 1. A. Lineage tracing (BdLT-Seq) was performed in the HA1ER clonal system. Data obtained from the founder clonal population (F12) and 2 subclones (1F8 and 1C9) is depicted as chord diagrams representing transcriptome state dynamics for cells belonging to a particular state/cluster at the beginning of tracing and their divergence after 4 days. Detected clusters are depicted (C0 to C12) and integrate the collapsed behaviour of all cells analysed that belong to each gene expression state. Origin clusters are shown in different colours (Day 0) and chords represent the endpoint cluster association (Day 4). **B.** Conceptual toy diagram depicting that BdLT-Seq data predicts that some clones will become enriched in a defined/reduced number of states as verified experimentally in C. **C.** The founder clone from the HA1ER (F12) and divergent subclones (1F8, 1C9, 2A6 and 1D12) were subjected to scRNA-Seq. UMAP plots represent transcriptome states and their divergence among analysed subclones. Individual states are depicted in colour and numbered. **D.** HA1ER clone 1F8 was subjected to immunocytochemistry for activated signalling pathways coupled to RNA-FISH targeting DPP4-DT lncRNA. The upper panel depict individual channels (DAPI, pERK1/2 and DPP4-DT) and a merged image (pERK1/2/DPP4-DT) whilst the lower panels depict merged images for DPP4-DT/pBRAF, DPP4-DT/pPI3K, DPP4-DT/pRAC1, DPP4-DT/pAKT and DPP4-DT/pMEK1/2. Inset is displayed for the upper panel.

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Bianca Blochl and Maxi Portal

(Figure 1D). These agregates co-segregate with phenotypic divergence, thereby pinpointing to a potential molecular device underlying phenotypic heterogeneity by modulating cell plastcity through signal integration (Signal Integration Portals - SIP). Indeed, altering the levels of diverging lncRNAs results in the redistribution of SIP-associated signalling cascade components within the cytoplasm, which is accompanied by a wide-scale transcriptome remodelling and shifts in phenotypic output. Therefore, we hypothesize that the repertoire of divergent lncRNAs present in each cell and their interaction with differentially expressed/localized IDR-proteins does facilitate the formation of membraneless condensates which depending on their molecular composition, will nucleate distinct signaling cascades in time and space, thus orchestrating phenotypic outcome in response to biological and/or therapeutic cues.

It is our hope that, due to the universality of our findings, our discoveries may prompt a new biotechnological revolution where biological control would move beyond genetic manipulation to finally harness the reprograming potential of the non-genetic compartment paving the way for the development of a new generation of anti-cancer agents.

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