Real-time visualization of the inception of drug tolerance in single melanoma cells

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***Short Abstract* — Drug resistance is a major problem in cancer therapy. However, little is known about how an initially drug-sensitive population persists in the presence of drug to eventually re-enter a proliferative state in which cells can acquire *bona fide* drug resistance mutations. Here we use long-term single-cell time-lapse microscopy and cell tracking to uncover cell-to-cell variability in signal transduction that enables some cancer cells to evade drug treatment. Our results implicate rapid signal rewiring events in the incomplete response of cancer cells to drug, and identify means to reduce heterogeneity in drug response.**

I. PURPOSE

SPONTANEOUS genetic mutations allow an initially drug-sensitive population of cancer cells to acquire a drug-resistant phenotype. However, little is known about how drug-sensitive cells first evade drug action and survive in the presence of drug, referred to as 'drug tolerance', a crucial step on the road to resistance. To better understand the timescale of the inception of drug tolerance and the heterogeneity within the drug-tolerant population, we combined single-cell time-lapse microscopy and MATLAB-based automated cell tracking to monitor Dabrafenib-treated melanoma cells harboring a BRAFV600E mutation.

II. RESULTS

By monitoring single melanoma cells expressing a live-cell CDK2 activity sensor that marks the proliferation-quiescence decision over the first five days of treatment, we discovered that the majority of the cells stop proliferating and remain quiescent for the duration of the drug treatment while a subset of cells escape drug action and occasionally divide in drug. These 'escapees' rapidly revert to the drug-sensitive state upon drug withdrawal, clearly implicating a non-genetic mechanism enabling proliferation in drug.

We further find that Erk is reactivated specifically in escapees several hours prior to cell-cycle re-entry, whereas Erk activity remains low in non-escapees. Consistently, co-treatment with a Mek or Erk inhibitor further inhibits Erk activity and nearly eliminates the escapee population, indicating that MAPK pathway reactivation is required for re-proliferation and that Erk activity is not fully suppressed by Dabrafenib treatment alone. This finding reveals the molecular underpinnings of the benefit of using combination therapy over the mono-therapy in melanoma patients. Profiling the escapee population by single-cell RNA-seq reveals that escapees rely on multiple mechanisms to re-proliferate in Dabrafenib, and that blocking each mechanism reduces the escapee population.

III. CONCLUSIONS

Together, our findings suggest that rapid signaling pathway rewiring in the first few days of drug treatment drives occasional cell cycling in drug, which enables the acquisition of genetic mutations and permanent drug resistance.

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