Functional immunoassay for evaluating drug sensitivity and ABC transporters' expression in patient-derived non-small cell lung cancer cells

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Non-small cell lung cancer (NSCLC) treatment faces challenges due to complex cancer cell interactions and tumour microenvironment (TME) dynamics, as well as the role of ATP-binding cassette (ABC) transporters in multidrug resistance (MDR). This study developed an immunofluorescence assay to evaluate cytotoxicity, selectivity, and ABC transporters' expression using the ImageXpress Pico high-content imager and CellReporterXpress 2.9 software. Primary patient-derived NSCLC cells were cultured for 2-3 weeks (short-term) and 6 weeks (long-term) and treated with cisplatin, carboplatin, etoposide, and gemcitabine at clinically relevant concentrations. We distinguished epithelial cancer cells from mesenchymal non-cancer cells with an antibody cocktail for cytokeratin 8/18. We also analyzed the expression of ABC transporters (ABCB1, ABCC1, and ABCG2) using specific antibodies. Results showed that prolonged culture increased the proportion of cancer cells while decreasing stromal cells. More extended culture conditions led to significantly higher baseline ABC transporters' expression and reduced sensitivity to the tested chemotherapeutics. This functional immunoassay provides insights into NSCLC sensitivity to anticancer drugs and potential treatment outcomes based on ABC transporters' expression. The study highlights significant differences between short-term and long-term culture models, emphasizing the need to choose appropriate culture durations for *ex vivo* drug screening.