Label-free Raman imaging for treatment response monitoring in patient-derived head and neck tumor slice cultures

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In modern oncology, the selection of effective therapies still presents significant challenges: tumor intrinsic complexity, high heterogeneity and dynamic behavior enabling adaptation and the development of resistance mechanisms strongly contribute to the impairment of treatment success rates. The possibility of monitoring therapy-induced effects is therefore of critical importance to enable timely, patient-tailored treatment adjustment, avoid unnecessary exposure to the toxicity associated with most anti-cancer therapies and ultimately improve patient prognosis. In this framework, Raman spectroscopy emerges as a promising technique, offering label-free, non-destructive, and chemically specific insights into biological samples. This study presents a novel methodology for chemical imaging of patient-derived head and neck tumor slice cultures.

Resected tumors were sliced in 350 µm-thick sections and cultured *in vitro*, aiming at reproducing the 3D tumor complexity. Programmed death ligand 1 (PD-L1), a membrane protein used as a target for many immunotherapeutic strategies, was administered for up to 4 days, to probe the intrinsic immune response of the model. Then, tumor cultures were further cut in 12 µm-thick slices, formalin-fixed and stored at -80 °C. A custom-built spontaneous Raman system was used for collecting Raman hyperspectral maps (120×120 pixels) with a scanning step size of 5 µm (total area covered of 600 μm^2) and 500 ms pixel dwell time. A 660 nm excitation laser light was focused on the sample with a 50× objective, with a resulting power at the sample plane of 35 mW, and signals from a spectral window comprising both fingerprint and CH regions (600-3400 cm^{-1}) were collected and analyzed.

Four patient-derived slice cultures were investigated, for a total number of more than 200 Raman maps (about 3M spectra). The full dataset was preprocessed with a unique pipeline to ensure data harmonization, including cosmic rays removal, substrate removal, baseline correction and normalization. Preliminary k-means clustering analyses discriminated tumor tissue from the lipid-protein ratio in the CH spectral region (2800-3050 cm^{-1}). In the fingerprint region, the collagen component (1250 cm^{-1}) accurately targets the tumor stroma, while a higher lipid and nucleic acids content (1314 cm^{-1} and 1339 cm^{-1}) was observed in the tumor regions. Immune response was analyzed via principal component analysis, comparing both PD-L1-treated and control models belonging to three patients. Time-dependent biochemical changes have been revealed, consisting in particular in an increased protein and DNA content and a concomitant reduction in lipid-associated Raman signatures in PD-L1-treated tumor tissue. These results are compatible with the expected suppression of the immune system response in presence of PD-L1 treatment, possibly leading to a consequent higher proliferation of cancer cells.

These early findings support the validity of Raman spectroscopy and the adopted methodology for the characterization of *ex vivo* patient-derived slice cultures and the monitoring treatment-induced biochemical changes over time, highlighting its potential as a tool for therapy assessment in oncology.