Detection of p53 aggregates in circulating tumour cells of patients with small cell lung cancer

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Background

TP53 mutations are found in 75-90% of small cell lung cancer (SCLC) patients. Mutations can make p53 prone to misfolding and the formation of oligomers with amyloid-like behaviour. The presence of p53 aggregates was already demonstrated in cancer cell lines, xenografts, as well as patient tissues and correlated with loss of p53 function and gain of toxicity. Moreover, evidence of prion-like cell-to-cell transmission has been shown in-vitro and in xenografts, suggesting p53 aggregates are a novel player in oncology.
The two common markers for CTC identification are the cell surface epithelial cell adhesion molecule (EpCAM) and cytokeratins (CK). However, EpCAM and CK are not expressed in some tumours and can be downregulated during epithelial-to-mesenchymal transition [1]. In order to improve CTC detection additional cancer markers are needed. Here we investigated the presence of p53 aggregates in circulating tumour cells (CTCs) in patients with SCLC using a novel proximity ligation assay.

Methods

To evaluate the performance of the method, GLC4, NCI-H526 and DMS53 cells were mixed with PBMCs isolated by density gradient centrifugation using BD Vacutainer® CPT™ Cell Preparation Tubes (Becton Dickinson, USA) from a healthy donor blood. The cells were fixed and immobilized on a glass slide. After permeabilising the cells, amyloid p53 was detected using a novel proximity ligation assay (PLA) based on proprietary technology from Navinci Diagnostics (Uppsala, Sweden). Subsequently, an immunofluorescent (IF) staining of cytokeratins (CK) was performed to identify cancer cells. Leukocytes were stained using an anti-CD45 antibody. The cell nuclei were stained with DAPI. The stained cells were analysed using the ALS CellCelector™ platform (Automated Lab Solutions, Jena, Germany).
For the analysis of CTCs, peripheral blood was taken after written informed consent from patients diagnosed with small cell lung cancer (SCLC). The blood samples were processed using the microfluidic Parsortix™ system (Angle plc, UK) to enrich CTCs. The cells were fixed and immobilized on a glass slide. Again, the PLA followed by IF was performed and the cells analysed using the ALS CellCelector™ platform.

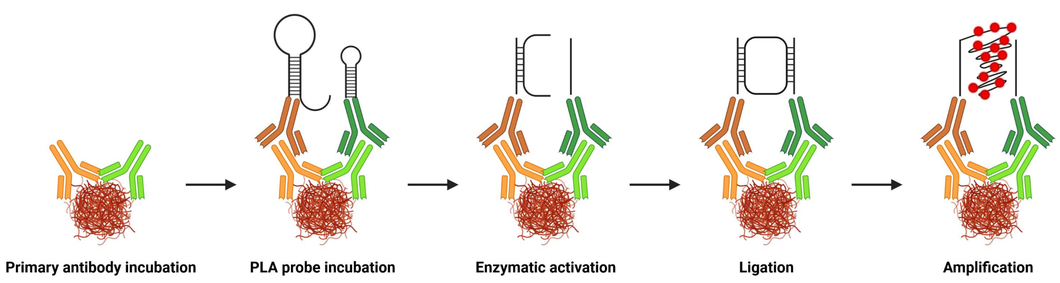

Figure 1: To detect p53 aggregates, a p53 antibody was combined with the amyloid specific A11 antibody. The primary antibodies were detected by secondary antibodies conjugated to proprietary oligonucleotides. After an enzymatic activation, these oligonucleotides form a circular DNA strand if present in close proximity. The resulting DNA circle is amplified by rolling circle amplification (RCA). Then, the amplified DNA is detected by fluorophore-labelled detection probes, which hybridize to the complementary sequence. Figure created with BioRender.com.

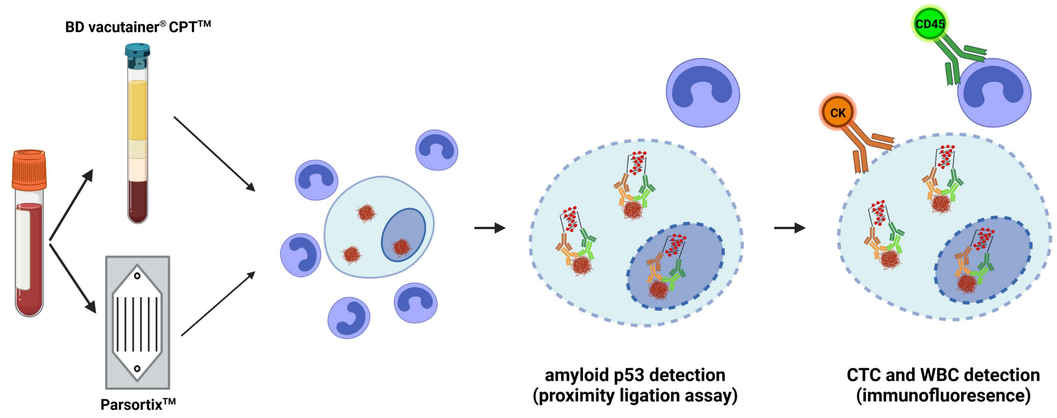

Figure 2: Workflow of CTC detection using PLA followed by IF staining. Figure created with BioRender.com.

Results

The novel PLA specifically detects p53 aggregates in SCLC cells but not in leukocytes

P53 aggregates could only be detected in SCLC cell lines but not in leukocytes. Notably, the GLC-4 did not express cytokeratin, but the cancer cells could still be identified as they were PLA positive. The NCI-H526 carries a *TP53* mutation leading to a premature stop codon and therefore, does not form p53 aggregates.

Table 1: SCLC cell lines analysed for the presence of p53 aggregates.

Cell line	*TP53* mutation status	Amyloid p53 expression	CK expression
DMS53	S241F	Positive	Positive
GLC-4	K132E	Positive	Negative
NCI-H526	E298Stop	Negative	Positive

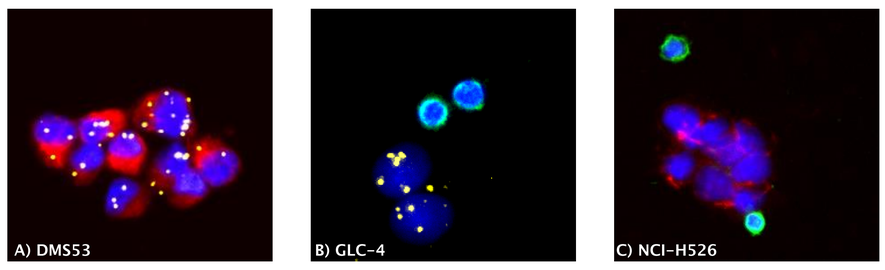

Figure 3: SCLC cells mixed with leukocytes isolated from a healthy donor. The PLA was combined with a subsequent immunofluorescence staining for CTC markers (cytokeratins) and CD45 for the identification of leukocytes. **A)** DMS53 cells were PLA and CK positive. **B)** GLC-4 cells were CK negative but could still be detected as tumour cells as they were PLA positive. **C)** NCI-H526 cells were CK positive but did not carry p53 aggregates.

p53 aggregates could be detected in CTCs from patients diagnosed with SCLC

p53 aggregates could be observed in single circulating tumour cells in two SCLC patient samples.

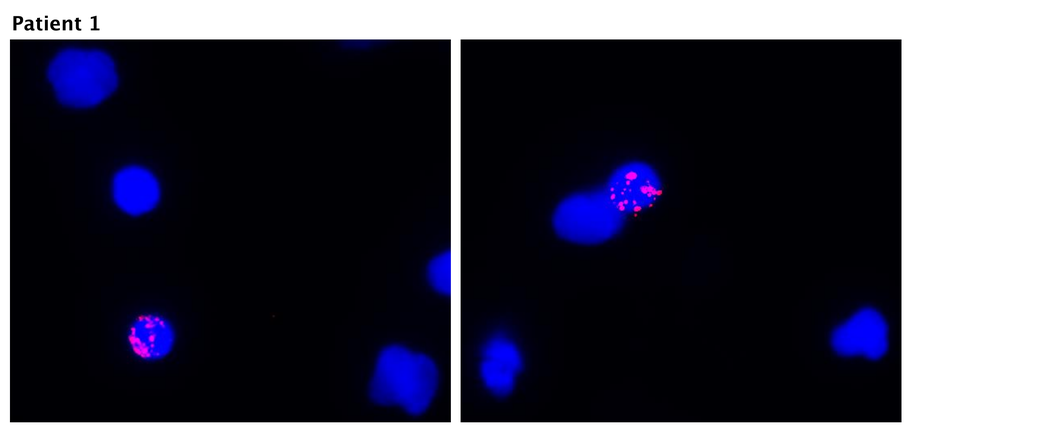

Figure 4: Representative images of p53 aggregates in circulating tumour cells of two patients with small cell lung cancer. Nuclear DNA was labelled with DAPI.

Conclusion

- As p53 aggregates could be detected in cancer cells only, their detection is a suitable marker to distinguish between cancer cells and leukocytes.
- Since the GLC-4 cell line did not show cytokeratin expression, but amyloid p53 could be detected, p53 aggregates are a promising marker for the detection of CK negative CTCs.
- For the first time, we could demonstrate that p53 aggregates are present in CTCs and that they are unique new marker for the detection of CTCs.
- Furthermore, the presence of p53 aggregates are likely a valuable biomarker for guiding and monitoring therapy, especially with p53 aggregates-targeting drugs.

References

1. Mikolajczyk, S.D., et al., Detection of EpCAM-Negative and Cytokeratin-Negative Circulating Tumor Cells in Peripheral Blood. Journal of Oncology, 2011. 2011: p. 252361.