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QUANTITATIVE ANALYSIS OF β-CATENIN/E-CADHERIN INTERACTION

Discover the power of functional biomarkers in tumour samples

WHITEPAPER

Quantitative analysis of β-catenin / E-cadherin interaction by QF-Pro®: Discover the power of functional biomarkers in tumour samples

β-catenin / E-cadherin complexes in cancer

Cell-cell junctions are essential regulators of tissue integrity, cell communication, and maintenance of epithelial cell polarity. They are established and maintained through various intercellular junctions, particularly adherent junctions. These junctions are primarily composed of cadherin-catenin complexes, which link the plasma membrane to the actin cytoskeleton. E-cadherin, expressed predominantly in epithelial cells, mediates homophilic interactions between adjacent cells through its extracellular domain, while its intracellular domain binds to β-catenin, anchoring the complex to the actin cytoskeleton. The intracellular interactions form a scaffold that orchestrates cell signalling from the plasma membrane. Altogether, these junctions form a tight, polarized epithelial layer essential for barrier and tissue functions[1].

The disruption of the β-catenin/E-cadherin complex is a hallmark of cancer. Downregulation and loss of E-cadherin are correlated with poor prognosis[3,4,5], and aberrant activation of β-catenin signalling is frequently observed[6,7].

Mechanistically, disruption of the β-catenin/E-cadherin complex leads to a decrease in cell-cell adhesion, epithelial-to-mesenchymal transition (EMT) and metastasis[2]. Loss or dysfunction of E-cadherin as well as mutations of β-catenin itself or of its regulatory pathways result in the uncoupling of the adherent junctions and dissociation of β-catenin from the membrane. β-catenin translocation to the nucleus acts as a transcriptional co-activator for genes promoting cell proliferation and invasion[2]. All these different mechanisms will contribute to oncogenesis by enhancing the invasive and metastatic potential of tumour cells.

These findings highlight the critical role of E-cadherin and β-catenin in maintaining epithelial tissue architecture and the consequences of their dysregulation in malignancies. Therefore, understanding the molecular mechanisms behind E-cadherin and β-catenin interactions is essential for developing therapeutic strategies aimed at restoring epithelial integrity, preventing metastasis, and ultimately improving cancer patient outcomes.

Consequently, assessing β-catenin/E-cadherin complex stability would greatly aid tumour prognosis by informing on possible therapeutic strategies aimed at restoring their normal function to inhibit tumour progression. This report details the quantitative analysis of the interaction between β-catenin and E-cadherin using QF-Pro® technology, as described overleaf.

QF-Pro® enables the visualization and quantification of β-catenin/ E-cadherin interactions

QF-Pro® is a technology capable of spatially quantifying functional proteomic events such as protein-protein interactions and protein post-translational modifications at a ≤10nm resolution. QF-Pro® basics are held in an improved and simplified methodology which combines resonance energy transfer and fluorescence lifetime imaging (FRET-FLIM), allowing the spatial mapping and precise quantification of protein interactions within fixed cell and tissue samples (Figure 1).

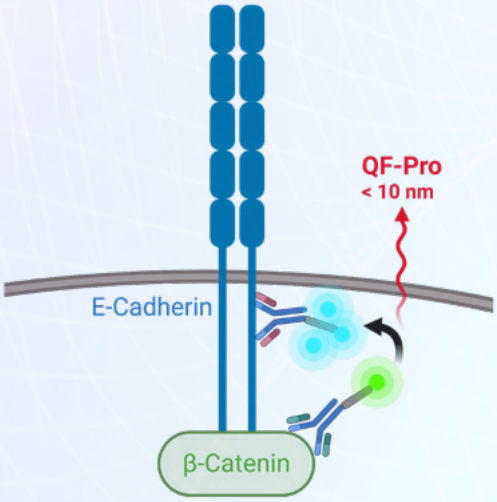

Figure 1: *QF-Pro® is a two-site assay that employs specific antibodies and fluorophore pairs to detect the proteins of interest. Precisely quantifying the decrease in the donor fluorophore lifetime (in this example β-catenin labelling) when it is at ≤10nm distance from the acceptor fluorophore (E-cadherin labelling) allows the calculation of a QF-Pro® score. The QF-Pro® scores provide the quantitative values allowing the comparison of functional proteomic events – such as complex formation as pictured - in various samples.*

Crucially, the precise measurement of the QF-Pro® signal in FFPE (formalin fixed paraffin embedded) samples is made possible through signal amplification of the acceptor fluorophore. This amplification allows for QF-Pro® signal to be measired and discerned from the intrinsic auto-fluorescence of the tissue samples.

QF-Pro® permits not only the subcellular visualization of β-catenin and E-cadherin expression but also the mapping and quantification of their interaction in different models such as cell lines (Figure 2) and FFPE-tissue (Figures 3 and 4). In all the images the expression of the proteins of interest are seen in green and cyan and the localisation of the interaction can be seen in red. To assess the interaction in a simple model, β-catenin/E-cadherin interaction was analysed in confluent epithelial cells (Figure 2). As expected, the localisation of β-catenin/E-cadherin interaction (red) was observed in the intercellular junctions.

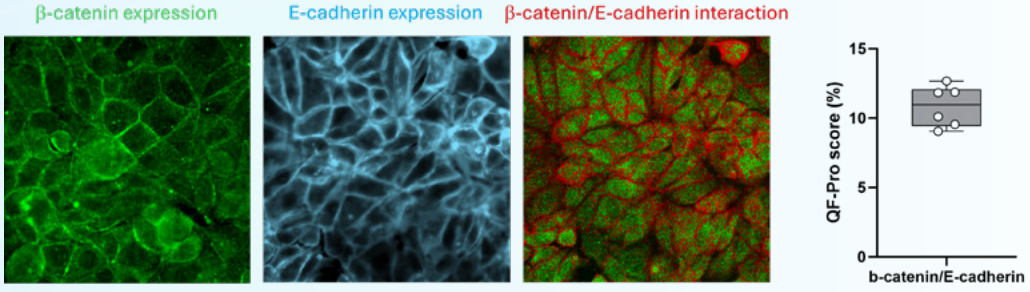

Figure 2- Quantification of β-catenin and E-cadherin interaction in MCF-7 cells: *MCF-7 cells were cultured to confluency and fixed with 4% PFA. Cells were labelled with primary antibodies recognizing β-catenin and E-cadherin. Specific QF-Pro® secondary reagents binding to primary antibodies, enable the visualization of β-catenin (green) and E-cadherin (cyan) distribution as well as their interaction (red). The box plot shows the average interaction of β-catenin/E-cadherin (in %) from different fields of view (dots).*

β-catenin / E-cadherin interaction in NSCLC

In lung cancer, a role for a dysfunctional cell-cell adhesion was shown in tumour progression and more specifically, the analysis of E-cadherin and β-catenin expression provided important evidence on which to base treatment in the clinic[8].

Given that clinical basis, this work assessed β-catenin / E-cadherin interaction state in two patients with non-small cell lung cancer (NSCLC) (Figure 3). Using the QF-Pro® assay, it was observed that Patient 1 (stage II) had a higher QF-Pro® score compared to Patient 2 (stage III). Measurement of different regions of the NSCLC tissues using the technology revealed the variability of the interaction.

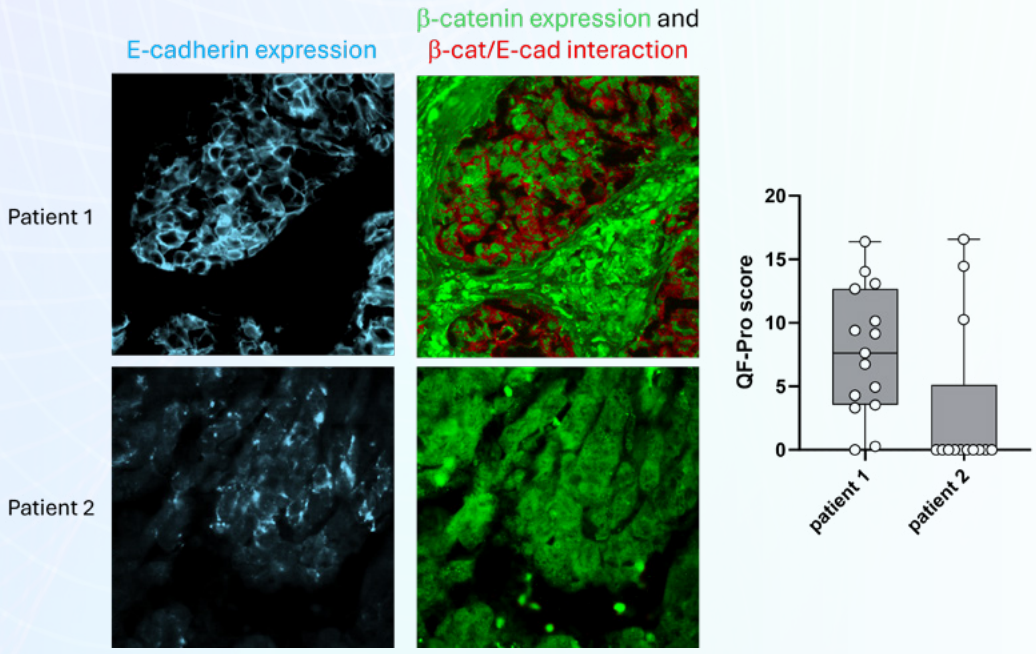

Figure 3 - Interaction of β-catenin and E-cadherin mapped with QF-Pro® in NSCLC patients: Images on the left show E-cadherin expression. Images on the right show the interaction (red) and the expression of β-catenin. The graph shows the interaction state of β-catenin/E-cadherin in two NSCLC patients measured by QF-Pro Score.

β-catenin / E-cadherin complex in Breast Cancer

In breast cancer, alterations in E-cadherin and β-catenin complexes through reduced membranous expression critically influence tumour development and metastatic behaviour, correlating with higher tumour grade and decreased survival rates[9]. The loss of E-cadherin is associated with EMT, while aberrant β-catenin activity amplifies oncogenic signalling pathways, with nuclear accumulation of β-catenin associated with poor prognosis[12].

β-catenin/E-cadherin downregulation leads to increased cellular motility and invasiveness[10]. This phenomenon is particularly evident in invasive lobular carcinoma (ILC) of the breast, where E-cadherin loss is a hallmark, often due to mutations or epigenetic silencing of the CDH1 gene[11].

Therefore, this work analysed the interaction of β-catenin and E-cadherin in a panel of 10 breast cancer patients using the QF-Pro® assay. All patients had invasive ductal carcinoma except for Patient 7, who had invasive lobular carcinoma. Figure 4A shows the heterogeneity of the E-cadherin expression (cyan) on 3 different patients, and specifically the loss of expression evident in the lobular carcinoma (Patient 7). In addition, a variability in the β-catenin distribution (green) which shows either membranous or perinuclear localisation was also observed. Finally, varying interaction states (red) can be observed across patients, along with different values of the calculated QF-Pro® scores. Remarkably, in all 10 patients, the level of β-catenin/E-cadherin interaction was dependent on the localisation of β-catenin (Figure 4B). In the patients where the expression of β-catenin was membranous (such as Patient 2), there were higher QF-Pro® scores (Figure 4B, red dots). Conversely, when β-catenin was mostly found in the cytoplasm with a perinuclear accumulation (e.g., Patient 9), the interaction was reduced (Figure 4B, green dots).

As expected, the downregulation of E-cadherin expression was correlated with a decrease in β-catenin/E-cadherin interaction (Figure 4C). However, many patients with significantly different levels of E-cadherin showed no interaction (red rectangle), while patients with similar E-cadherin expression either presented no interaction or a significant QF-Pro® score (purple rectangle). Overall, the only approximative correlation between QF-Pro® scores and E-cadherin expression suggests that E-cadherin expression would not accurately predict the engagement with β-catenin and hence would not properly reflect the activation of the downstream signalling.

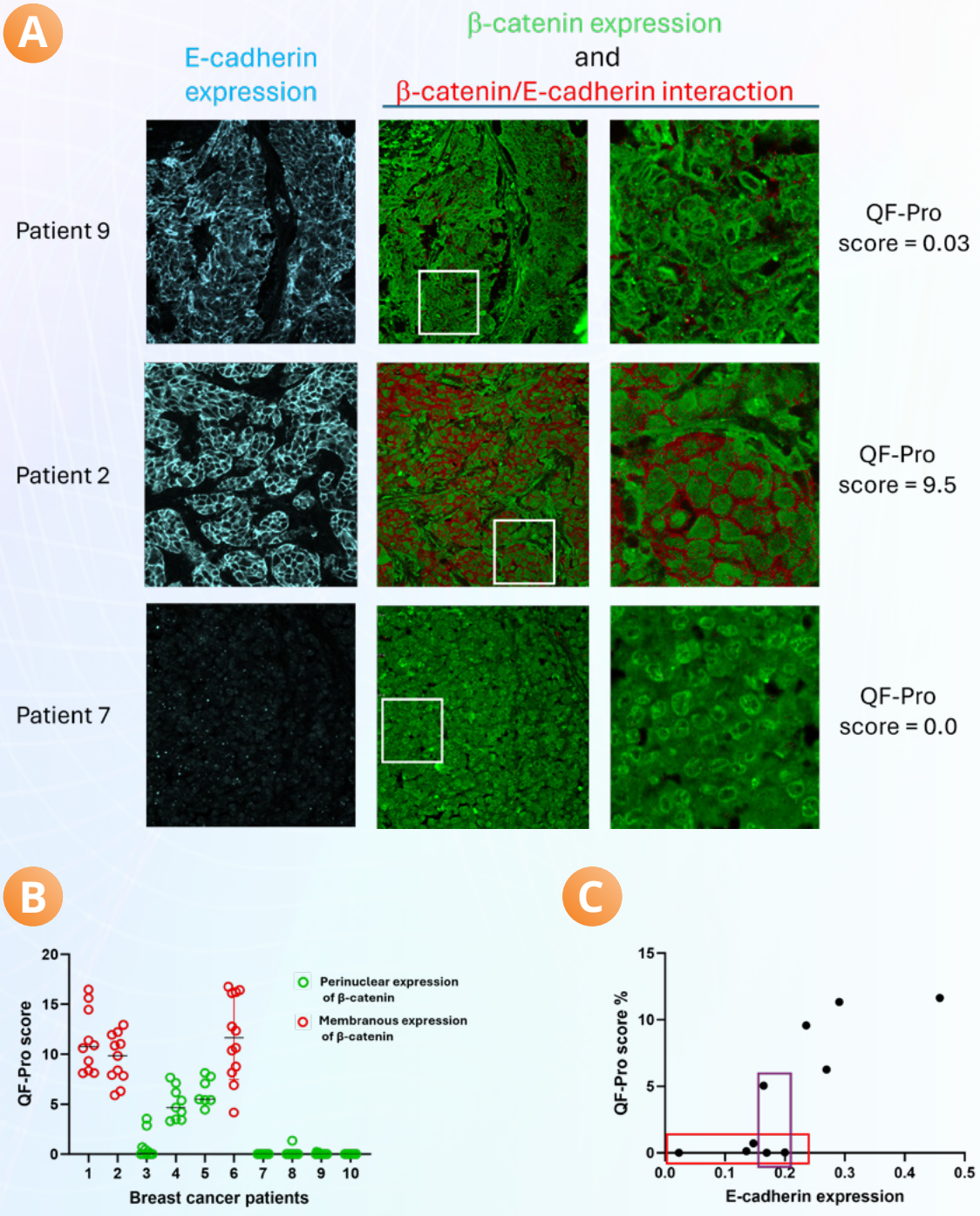

Figure 4 - Interaction of β-catenin and E-cadherin in breast cancer patients visualized and quantified by QF-Pro®:

A) *Representative images of patients with various expressions of E-cadherin (left panels, cyan). Patients 9 and 2 present invasive ductal carcinomas while patient 7 presents an invasive lobular carcinoma. Images show the expression of β-catenin (green) as well as the interaction of β-catenin with E-cadherin (QF-Pro® scores, in red). Right images show the amplified area highlighting the different distribution of β-catenin (white box).*

B) *QF-Pro® scores of a panel of 10 breast cancer patients show the variability of β-catenin/E-cadherin interaction dependent on the localisation of β-catenin. Higher QF-Pro® scores can be seen in patients with membranous expression of β-catenin and lower QF-Pro® scores in patients with perinuclear expression of β-catenin.*

C) *E-cadherin expression and QF-Pro® scores relationship.*

Conclusion

The analysis of functional biomarkers in FFPE tissue samples is particularly difficult to assess with traditional methods like immunohistochemistry or immunofluorescence, and do not generally provide accurate quantitative measurements of the biomarkers function. Here the interaction of β-catenin and E-cadherin was precisely quantified and mapped using QF-Pro® in cells and FFPE tumour samples. The amplification of the signal allowed for the accurate measurement of this functional biomarker in formalin-fixed tissues, which are notoriously hard to work with due to their high intrinsic autofluorescence. The data showed that the expression levels of the 2 proteins did not necessarily correlate with the level of interaction of the complex, highlighting the limitation of solely measuring protein expression to predict the activation of the downstream signalling. Obtaining robust continuous quantitative values with QF-Pro® allowed for the detection of subtle variations in the intra- and inter-heterogeneity of the patients' interaction profiles, complementing β-catenin subcellular localisation and E-cadherin expression to inform on the signalling output. Ultimately, obtaining quantitative functional biomarker readouts (QF-Pro® scores) from large patient cohorts could provide the necessary numerical basis to derive cut-off values of interactions essential to inform the selection of effective cancer treatments for patients in clinical settings.

Contact Us

Find out more information about our QF-Pro® services at:

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