Proteomic Identification of Tissue Degradation Indicators for Tissue Quality Assessment

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INTRODUCTION

Analysis of human surgical specimens is important for diagnosis and treatment of disease. Analytical methods include – among others – histologic stains as well as DNA, RNA and protein analysis. However, variable degrees of molecular degradation typically occur in human surgical specimens before the degradation process is effectively suspended via fixation or freezing techniques. Since degradation is known to affect analytical results, a universal test for biochemical integrity would be of great interest for specimen-based studies. We therefore initiated an investigation to identify protein markers for tissue degradation assessment.

MATERIALS & METHODS

1. Cell lines and clinical materials: 4 human cell lines: 293T, MDA-MB-231 (HTB-26™), Jurkat (TIB-152™) and LNCap (CRL-1740™), and 62 clinical specimens including autopsy specimens were used.

2. Cold Ischemic Time (CIT) – dependent tissue degradation model: We exposed human cell lines and surgical/autopsy specimens to defined periods of time at 23°C prior to specimen collection and procurement; this model will be referred to herein as experimental Cold Ischemic Time (CIT)-dependent tissue degradation model.

3. Proteomic comparison between specimens of different CITs: Using Two Dimensional Fluorescence Difference Gel Electrophoresis (2D DIGE) in conjunction with LC/MS/MS and MALDI-TOF/MS, we performed comparative proteomic analyses on the cell lines at different CIT exposures and proteins that undergo CIT-dependent degradation were subjected to MS sequencing.

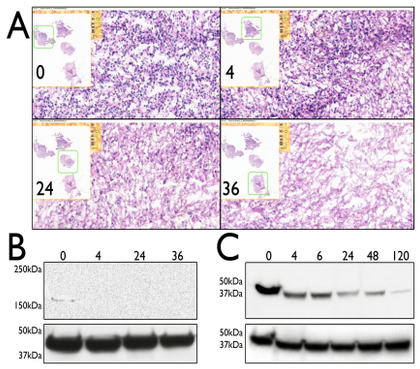
4. Validation of proteomic findings using Western analysis: Duplicated mass spectrometry-based protein sequencing generated two independent data sets. Proteins with consensus IDs from both MALDI and LC platforms were selected for antibody-based validation studies using One Dimensional (1D) and Two Dimensional (2D) Western blotting following standard procedures.

5. Generation of tissue degradation reference curve: Western analysis verified that the degradation of several proteins was strongly associated with the impact of CIT. Therefore, these proteins may serve as Tissue Degradation Indicators (TDIs). Via quantitative analysis on Western data, TDI-based tissue degradation reference curves were generated for retrospective degradation assessment on incoming tissues.

Acknowledgement: Support from Dr. Morrow's laboratory in spectrin studies.

RESULTS

1. CIT Impact on diagnostic features of surgical specimens.



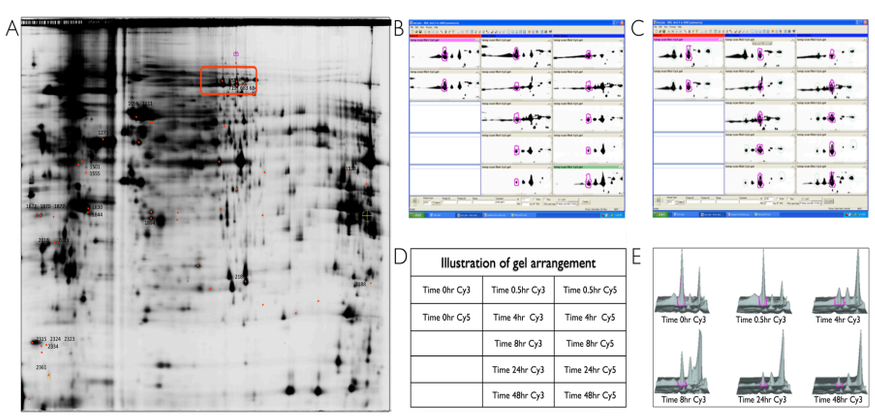
Four equal fractions of an intracranial meningioma were randomly assigned to 0-hour, 4-hour, 24-hour, and 36-hour CIT exposure and embedded in a frozen tissue array.

A: H&E. Significant morphological changes are observed during degradation process.

B: Western analysis. Using proteins extracted from above tissue array, 170 kDa EGFR expression was detected in Time 0 tissue but completely undetectable in other tissue pieces with longer CIT exposure.

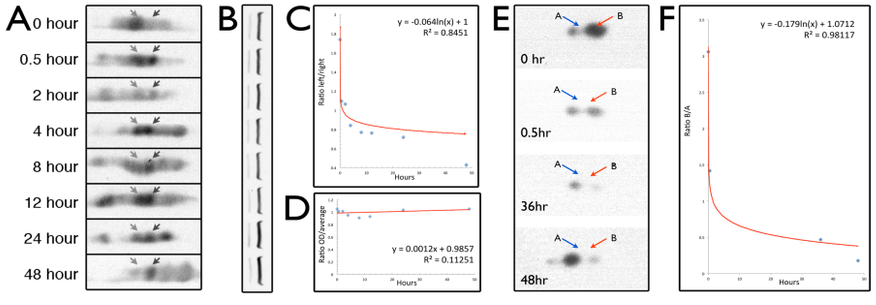
C: Western analysis. p53-positive 293T cell line were used to observe p53 changes during tissue degradation. Significant p53 degradation was observed during CIT exposure.

2. 2D Difference gel electrophoresis (DIGE) proteomic profiling and comparison.



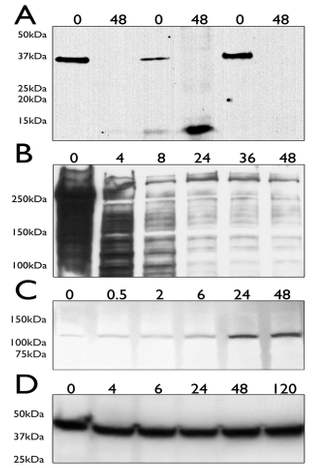
A: Representative 2D DIGE images obtained from Typhoon scanner using 293T cells (CIT = 0 hour). Red-framed region in all 12 experimental groups is demonstrated in high resolution with Decyder software (**B&C**). **B**: the same protein spot is pink-circled in all 12 gels (gridded windows). **C**: a neighboring protein spot is highlighted in 12 gels. **D**: illustration of gel layout in **B&C**. Quantitative decrease of protein spot in B and quantitative accumulation of protein spot in **C** are observed during CIT exposure. **E**: a reciprocal change between protein spot in **B** (highlighted peak) and protein spot in **C** (right most peak) is illustrated in a three dimensional format.

3. 2D Western demonstrates TDIs' isoelectric point changes during degradation.



MS analysis identified 27 Tissue Degradation Indicator (TDIs). Western analysis was used for validation of TDIs **A**: two EEF2 (example TDI) isoforms show reciprocal changes during tissue degradation (CIT hours are labeled on the left). Quantitative ratio between the left spot (light arrow) and the right spot (dark arrow) is associated with respective CIT impact, as indicated by the logarithmical-trend-line in **C**. In contrast, **B&D**: using identical cell lysate under identical experimental condition, 1D Western blot is not able to distinguish EEF2 changes during tissue degradation. **E&F**: a reciprocal change of the two isoforms (spot A and B, indicated with blue and red arrow, respectively) of protein PPP2C1 is observed during tissue degradation.

4. 1D Western blotting demonstrates TDIs' quantitative changes during degradation



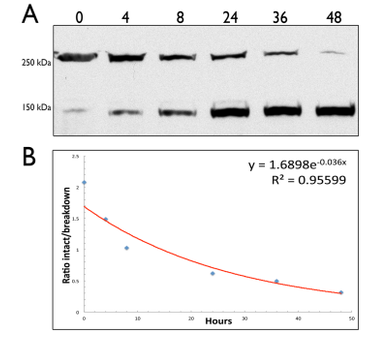
A: quantity of B23 protein decreases during CIT exposure in three meningioma specimens (tumor 1: 1&2 lane, tumor 2: 3&4 lanes, and tumor 3: 5&6 lanes).

B: commercially available anti-AHNAK antibody cannot yield a discrete immune-signal of this super-sized protein (630 kDa) in a meningioma specimen but reacts with protein products of smaller molecular size (likely AHNAK breakdown products). A relatively intact pattern of AHNAK can still be observed in early CIT exposure while this intact pattern fades in later stages of tissue degradation.

C: Western analysis demonstrates an accumulation of ubiquitin-activating enzyme E1 (UBA1) at its proper 110-kDa size during 293T cell degradation.

D: beta-actin is strongly and continuously expressed at all time points observed.

5. Reciprocal changes of alpha II spectrin during degradation

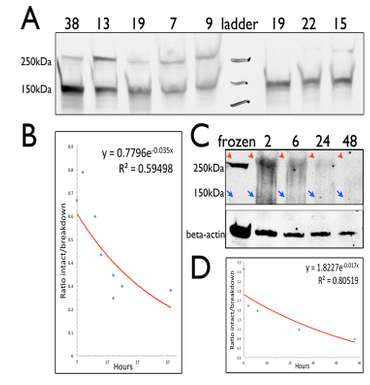


1D Western blot demonstrates the dynamic conversion between the intact form (285kDa) and its breakdown product (150kDa) of alpha-II spectrin during tissue degradation in a meningioma specimen.

A: Western analysis.

B: exponential trend line indicates the strong association between the spectrin intact/ breakdown ratio and the CIT impact.

6. Spectrin may serve as a TDI for autopsy and FFPE tissues.



A: Western blot to reveal spectrin breakdown during degradation in autopsy tissues. Post mortem intervals are labeled.

B: association between spectrin breakdown ratio and PMI.

C: degradation-induced spectrin breakdown in FFPE tissues. Tissue fractions of a meningioma specimen were exposed to CITs followed by 12-hour formalin fixation and routine paraffin embedding. Western analysis shows that spectrin is difficult to be detected as clear immuno-signals in FFPE tissues (upper panel); whereas low molecular weight beta-actin can be detected (lower panel in **C**).

D: using the blurry immune-signals at expected spectrin sizes (red arrows indicate the intact spectrins and blue arrows indicate spectrin breakdowns), we can still determine the correlation between spectrin breakdown and the CIT impact in these FFPE tissues.

CONCLUSIONS

Quantitative examination of TDI degradation can represent the overall stage of specimen degradation. The combination use of TDIs may yield a potential transformative platform dedicated to quality control in clinical specimen analyses.