Exploring missing proteins expression in gastric cancers

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# Abstract

**Background:** Missing proteins (MPs) are proteins lacking sufficient supporting evidence from mass spectrometry (MS) or other direct protein methods (Baker et al. 2017). The number of MPs has been constantly reduced due to the development of new detection techniques and through efforts from the growing community (Omenn et al. 2019). Gastric cancer accounts for 1.5% of all newly-diagnosed cancers in the united states (American-cancer-society 2022) and exploring the fingerprints of proteins, including MPs, can help us to better understand gastric cancer.

**Objective:** To explore the distribution characteristics of expressed MPs in gastric-cancer primary cell samples and evaluate how it associates with non-missing (regular) proteins.

**Methods**: A total of 198 MPs were detected in 8 gastric-cancer primary cell samples. Normalized spectral abundance factors (NSAFs) (Paoletti et al. 2006) were calculated using MS spectral counts. Transcripts per million (TPM) (Conesa et al. 2016) were calculated using RNA-Seq count data from the same 8 samples and were matched with their NSAFs on the gene level to check the association between proteomics expression and DNA expression for both MPs and regular proteins (Edfors et al. 2016).

**Results**: Six of the eight samples showed a similar level of MP and regular protein detection (min-max range: [18, 39] for MPs and [4,157, 6,119] for regular proteins). The proportion of protein products with RNA products was 54.70% and 58.04% respectively for MPs with and without the two potential low-profiling samples for their noticeably fewer detected proteins, which were 90.38% and 91.02% for regular proteins. The protein expressions of MPs showed a clear truncated pattern by lacking low-abundance expression indicated by gap region in the low-end of distribution. There is a significant linear association between protein expression and RNA expression for MPs (R=0.17, p = 0.029) and regular proteins (R=0.38, p<2.2e-16). Several MP genes, such as CTAGE1, were consistently detected with protein products and their RNA products.

**Discussion:** The highly-truncated expression distribution pattern of MPs could not be completely explained by the insensitivity of count-based-MS proteomics in low-abundance proteins (Lundgren et al. 2010) by seeing only a mild truncated pattern in regular proteins. The significant association between RNA-Seq and proteomics suggests the validity of our findings. The detections of MPs, such as Q9HC47 (CTAGE1), were supported by the clear association between proteomics and RNA-Seq data in gastric cancer and should be further explored their potential as biomarkers in gastric cancer.

Keyword: Mass spectrometry, Missing protein, Gastric cancer

Table 1. The proportions of proteins products with RNA products for missing proteins and regular proteins

|  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  |  | **Sample 1** | **Sample 2** | **Sample 3** | **Sample 4** | **Sample 5** | **Sample 6** | **Sample 7** | **Sample 8** | **Total** | **Total without low-profiling samples** |
| **Missing Proteins** | **RNA product (+)** | 8  (50.00%) | 39  (59.09%) | 24  (55.81%) | 20  (55.56%) | 22  (57.89%) | 18  (64.29%) | 7  (25.93%) | 25  (56.82%) | 163  (54.70%) | 148  (58.04%) |
| **RNA product (-)** | 8 | 27 | 19 | 16 | 16 | 10 | 20 | 19 | 135 | 107 |
| **Total protein products** | 16 | 66 | 43 | 36 | 34 | 28 | 27 | 41 | 298 | 255 |
| **Regular Proteins** | **RNA product (+)** | 3155 (90.43%) | 6028  (88.99%) | 6119  (91.02%) | 4730  (90.94%) | 5162  (92.20%) | 4157  (91.6%) | 3693  (85.17%) | 5465  (91.88%) | 38,509  (90.38%) | 31,661 (91.02%) |
| **RNA product (-)** | 334 | 746 | 604 | 471 | 437 | 381 | 643 | 483 | 4099 | 3122 |
| **Total protein products** | 3,489 | 6,774 | 6,723 | 5,201 | 5,600 | 4,538 | 4,336 | 5,948 | 42,608 | 34,783 |

**\* Sample 1 and Sample 7 were considered as potential low-profiling samples for their noticeably fewer detected proteins.**

Graphical user interface

Description automatically generated

Figure 1. The scatter plot of protein-RNA-product matched pairs. (a): All protein-RNA pairs for missing proteins. (b): protein-RNA pairs with TPM > 0 and NSAF > 0. (c): All protein-RNA pairs for regular proteins. (d): All protein-RNA pairs for regular proteins with TPM > 0 and NSAF > 0.

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