Glypican-3 immunohistochemistry precision, validation, and prevalence in selected solid tumors to identify target populations for CAR T cell therapy

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Background

Glypicans are membrane-bound heparin sulfate proteoglycans, known to stimulate or inhibit growth factor activity and are expressed during development in a cell- and tissue-specific manner. Glypican-3 (GPC3) is an oncofetal tumor antigen that is an attractive target for chimeric antigen receptor (CAR) T cell therapy due to its highly restricted expression on normal tissue and high prevalence in several adult and pediatric solid tumors. GPC3 is involved in regulation of cell proliferation and apoptosis in normal development during embryogenesis and expression is largely absent in normal adult tissues. Aberrant GPC3 expression is implicated in tumorigenesis.

Goal

To assess the validity of a GPC3 antibody clone in immunohistochemistry (IHC) and measure GPC3+ prevalence in selected solid tumors.

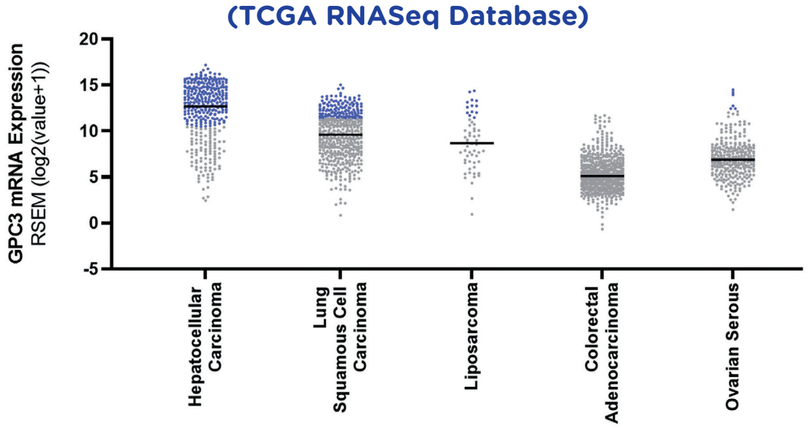
Methods

- We evaluated GPC3 transcripts in human normal and tumor tissue specimens from The Cancer Genome Atlas (TCGA) and the Genome-Tissue Expression Project (GTEx).
- GTEx: The RNA expression profile for GPC3 (ENSG00000147257.13) in normal human tissues was accessed from the Genome-Tissue Expression Project (GTEx) analysis release V8 (dbGaP Accession phs000424.v8.p2) on September 24, 2021 via the GTEx portal (https://gtexportal.org/home/). mRNA expression is reported as TPM and box plots are shown as the median and 25th and 75th percentiles. Points are displayed as outliers if values are greater than or less than 1.5 times the interquartile range.
- TCGA: RNA-sequencing profile of GPC3 expression in patient tumors from selected indications were generated in whole by the TCGA (The Cancer Genome Atlas) Research Network. Data were accessed using the cBioPortal for Cancer Genomics hosted by the Center for Molecular Oncology at Memorial Sloan Kettering. Data for selected indications was downloaded from cBioPortal on January 8, 2021 and visualized using GraphPad Prism 9 software. mRNA expression is reported as RSEM (batch normalized from Illumina HiSeq_RNASeqV2) (log2(value + 1))
- GPC3 IHC was performed on formalin fixed paraffin embedded tissues: normal tissue, hepatocellular carcinoma (HCC), squamous cell lung cancer (SCC), colorectal cancer (CRC), serous ovarian cancer (OC), Merkel cell carcinoma (MCC) and liposarcoma (LS). 5µm sections were stained using anti-GPC3 mouse monoclonal antibody clone GC33. Percent of tumor cells with GPC3 membrane and cytoplasmic staining at each intensity (0, 1+, 2+, 3+) was recorded and an H-score (range 0-300) was calculated as follows: H-score = 1*(%tumor cells with 1+ intensity) + 2*(%tumor cells with 2+ intensity) + 3*(%tumor cells with 3+ intensity). The cutoff for positive GPC3 expression was set at H score ≥ 30 in tumor cells stained with the GC33 IHC assay.
- For validation of antibody clone GC33 for GPC3 IHC staining as a lab-developed test, all tumor tissue samples were stained with GC33 or 1G12 (a GPC3 IHC antibody clone used as an in vitro diagnostic) and compared. GPC3+ cutoff was set as H-score ≥ 30 (GC33) compared to >20 (1G12). Specimens that showed consistent positive or negative results by both GC33 and 1G12 were considered true positive or negative and those with inconsistent results were indicated as false positive or false negative. Accuracy, sensitivity and specificity of GC33 was evaluated as observed true staining in total specimens stained, observed true positive staining in expected positive specimens and observed true negative staining in expected negative specimens respectively. Precision was determined by concordance of results from repeat staining of select specimens. The prevalence of GPC3 expression measured by IHC was also used to calculate the theoretical mRNA cutoff value from the TCGA data for GPC3+ indications represented in the database.

Results

- GPC3 mRNA was expressed at low levels predominantly in lung, adipose, tibial nerve, kidney and breast. (Figure 1)
- GPC3 (GC33) IHC of normal human tissue showed predominantly faint, cytoplasmic staining in a few tissues including heart, kidney and stomach and no expression in lung or breast (data not shown).
- HCC, SCC, LS, CRC, and OC showed high and varying levels of GPC3 mRNA expression in TCGA samples. A theoretical mRNA expression cutoff value was calculated as the mRNA expression value representing the prevelence of GPC3 expression in IHC for the given indication with the assumption that mRNA expression corresponds directly with protein expression levels. The cutoff values were HCC=1369 RSEM, SCC= 2675 RSEM, Sarcoma= 4126 RSEM; and OC 4486 RSEM; respectively. A cutoff for CRC could not be determined because the prevalence of GPC3 by IHC was <10%. (Figure 2)
- HCC, SCC, and myxoid/round liposarcoma (MRCLS) samples in the TMAs showed appreciable levels of GPC3 expression by GC33 IHC (H-score ≥ 30) with >20% prevalence (Table 1). No appreciable expression was observed in CRC (n=80) or OC (n=31) with <10% cases exhibiting an H-score ≥ 30. Additional IHC performed on 20-40 unique tumor samples for each of these indications: HCC, SCC, liposarcoma, as well as, MCC showed positive GPC3 staining (H-score ≥ 30) in >30% cases. GPC3 IHC using GC33 showed a higher prevalence of positive cases in myxoid/round cell LS (MRCLS) relative to other LS subtypes (15/45 vs. 8/75). HCC (n=110), SCC (n=73), MRCLS (n=45), and MCC (n=20) had a prevalence of 69%, 33%, 33%, and 70%; respectively. (Table 1 and 2, Figure 3)
- The GC33 assay was fully validated for use as a lab-developed test as it met all acceptable criteria (accuracy: 95%; sensitivity: 100%; specificity: 92%; precision: 100%) for each of these parameters in SCC, HCC, MCC, and LS specimens.

Figure 2



Legend: The blue data points represent those above the theoretical cutoff and the black line represents median mRNA expression for the indication.

Figure 1

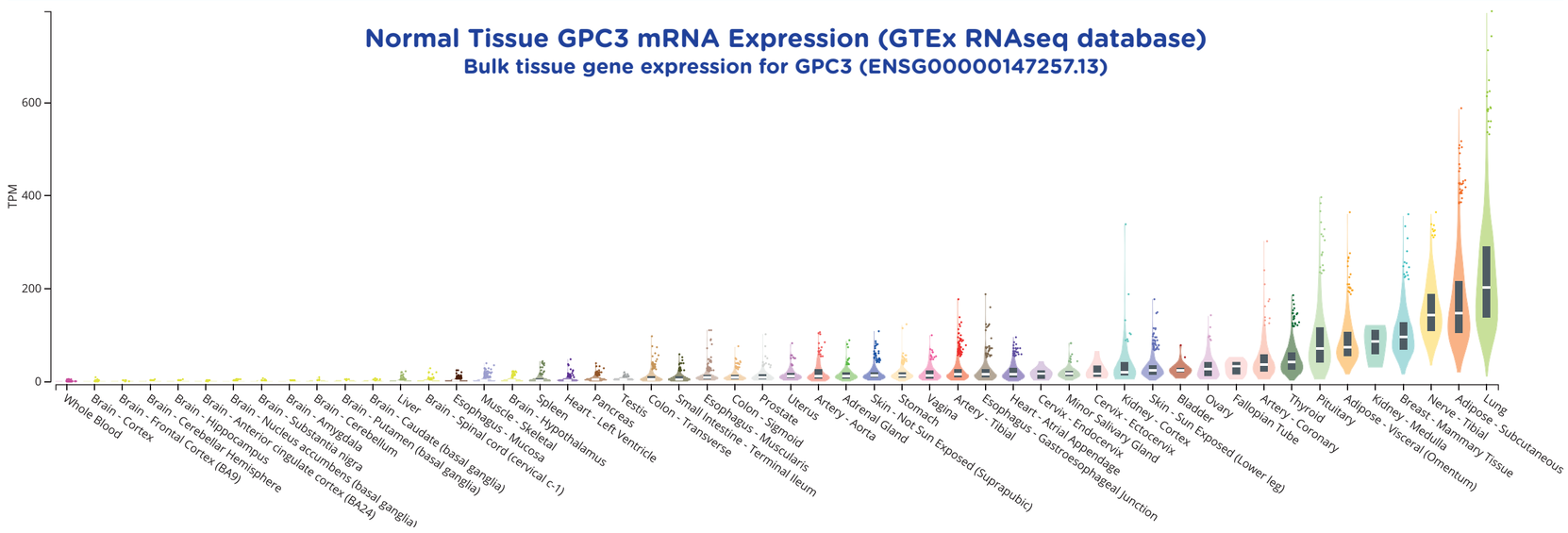


Figure 3

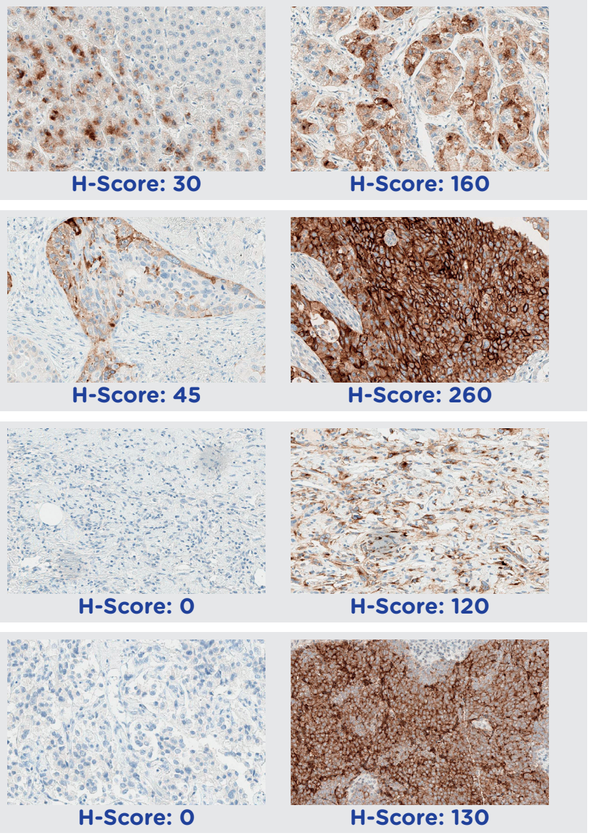


Table 1

TMA indication	Number of cases	Number of cases with H score ≥ 30
HCC	70	51
Lung cancer	75 (10 small cell undifferentiated carcinoma, 33 squamous cell carcinoma, 32 adenocarcinoma)	Only scored squamous cell carcinoma 9
Liposarcoma	80 (30 pleiomorphic liposarcoma, 25 cases of myxoid/round liposarcoma, 15 well-differentiated liposarcoma 10 other liposarcomas)	11 all liposarcoma 8 (myxoid/round liposarcoma only)
Ovarian cancer	77 (15 cases of cystadenoma 31 serous carcinoma, 6 mucinous adenocarcinoma, 10 endometrioid adenocarcinoma 15 others on ovarian cancer progression spectrum)	Only scored serous ovarian cancer 2 (serous ovarian cancer)
Colorectal cancer	80	0

Table 2

Indication	Number of cases	Number of cases with H score ≥ 30
HCC	40	25
SCC	40	15
Liposarcoma (MRCLS)	40 (20)	12 (7)
MCC	20	14

Conclusions

GPC3 has a high prevalence in selected tumors with low level expression in some normal tissues, making it an attractive target for CAR T cell therapy. The GC33 clone performs similarly to 1G12 and could be used for IHC screening for CAR T cell therapy. MRCLS has higher prevalence of GPC3+ expression relative to other LS subtypes.

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