SARS-CoV-2 infection associated cell entry proteins ACE2, CD147, PPIA, and PPIB as potential prognostic and infection biomarkers in neuroblastoma

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Abstract

Introduction: Although most children experience minor symptoms from SARS-CoV-2 infection, some develop complications including MIS-C. Neuroblastoma patients may be at higher-risk of infection. We hypothesized that ACE2, CD147, PPIA and PPIB are poor-prognosis and SARS-CoV-2 infection biomarkers in neuroblastoma. Results: In-depth gene-expression-dataset statistical analysis showed ACE2, CD147, PPIA and PPIB high-expression significantly associated with poor-prognosis neuroblastomas with amplified MYCN, unfavourable-tumors and patients older than 18 months of age. Low-expression was associated with the NTRK1-PTPN6-TP53 module, a good-prognostic marker. Conclusion: ACE2, CD147, PPIA and PPIB high expression is associated with poor-prognosis neuroblastomas. These patients may be at higher-risk of SARS-CoV-2 infection.

1. INTRODUCTION

SARS-CoV-2 viral infection has resulted in a global pandemic[1]. Severe infection elicits a hyper-inflammatory response and poses a risk to immunocompromised patients with pre-existing medical conditions[2].There is a need to understand the detrimental effects that SARS-CoV-2 has on neuroblastoma cancer patients.

Although most children experience minor symptoms from SARS-CoV-2 infection[3], some develop neurological complications including encephalopathy and seizures[4], or the life threatening MIS-C[3]. Neuroblastoma patients could be at a greater risk of infection as their immunocompromised status, as a result of chemotherapy, and specific molecular susceptibilities to the virus caused by cancer could increase infection risk[3,6]. Since SARS-CoV-2 contagion will continue in the future, it is critical to determine whether neuroblastoma patients are at higher-risk of infection.

Neuroblastomas are the most frequent neoplasms of infancy[5]. Patients are primarily diagnosed in the first 12 to 24 months of age[5] .Long-term survival with high-risk-type tumors is poor. Prognosis depends upon patient age and tumor biology[5]. Good-prognosis neuroblastomas (differentiated and favourable cytogenetics[5,7]), express the NGF receptor-tyrosine-kinase NTRK1[7,8], whereas poor-prognosis tumors (poorly differentiated, with SCA and MYCN-amplification[7,8]), express the neurotrophin receptor-tyrosine-kinase NTRK2[7,8]. On NGF-stimulation, NTRK1 is activated by tyrosine-phosphorylation[8]. This induces signalling promoting neuroblastoma cell differentiation[8]. Neuroblastomas display high-cell heterogeneity, which is reflected in their clinical presentation and poor outcomes[5,7,8].

We have demonstrated that TP53 represses PTPN6 phosphatase expression resulting in NTRK1-activation[9]. This type of NTRK1-activation is independently and significantly associated with 5-year RFS of children with

neuroblastoma[10] in the presence of MYCN-amplification, SCA and undifferentiated status[10]. This suggests that the NTRK1-PTPN6-TP53 module could be a predictive good-prognosis neuroblastoma biomarker[10].

ACE2, an enzyme belonging to RAS that cleaves Angiotensin I to Angiotensin II, behaves as the major SARS-CoV-2 cell-entry-receptor by binding the SARS-CoV-2 S protein[11]. ACE2 expression is decreased in breast-cancer and hepatocellular carcinoma[12,13]. Upregulation of the ACE2/Ang-(1-7)/Mas-axis inhibits breast-cancer cell-migration[14]. However, ACE2 upregulation is associated with poor-survival in lung-adenocarcinoma[15,16] and the ACE2/Ang-(1-7)/Mas-axis promotes migration and invasion of renal-carcinoma cells[17].Therefore suggesting that ACE2 plays a cancer specific role.

CD147, a cell-surface receptor[18], interacts with SARS-CoV-2 S protein to mediate viral infection[18]. Loss of CD147 inhibits increased SARS-CoV-2 viral load, and expression of CD147, in non-susceptible cells, facilitates viral-entry[19]. In breast- and colorectal-cancer high CD147 expression is associated with poor DFS[19]. CD147 is expressed in neuroblastoma exosomes[20] and high levels are associated with undifferentiated tumors[21].

PPIA and PPIB peptidyl-prolyl-cis-trans-isomerases[22], facilitate SARS-CoV viral-entry by interacting with CD147[23]. They interact with SARS-CoV nsp-1and are incorporated into the viral capsid and then released, enabling further virus binding to CD147 and subsequent infection of CD147-expressing cells[23]. They are overexpressed in glioblastoma and non-small-cell lung carcinoma[24]. PPIA correlates with poor outcome in gastric-cancer [24]. PPIB promotes cell-proliferation and invasion in non-small-cell-lung-cancer[25].

Our aim is to identify potential biomarkers that could be used as prognostic indicators and potential neuroblastoma treatment targets. We have hypothesised that ACE2, CD147, PPIA, and PPIB genes which are involved in SARS-CoV-2 viral cell-entry are potential biomarkers for neuroblastoma and SARS-CoV-2 infection.

2. RESULTS

2.1 ACE2, CD147, PPIA and PPIB association with neuroblastoma EFS

To assess whether expression of ACE2, CD147, PPIA, or PPIB have prognostic value, three neuroblastoma gene expression datasets were analysed. GEO database, accession number-GSE49711 with 498 samples of Agilent customized oligonucleotide microarray data and GSE62564 Illumina HiSeq 2000 RNA-Seq data from the same samples. TARGET, the third dataset, contains Affymetrix Exon-ST microarray data from 249 samples. Analysis was undertaken in 492 samples obtained from GSE49711 as those had *MYCN* amplification data. Although GSE49711 was used for the main analysis, results were confirmed with GSE62564 and TARGET datasets (Supplemental Methods). Clinical characteristics can be seen in Supplemental Table S1.

Multivariate analysis (Table 1) showed high and moderate ACE2 (HR= 1.48,P = 0.05 and HR= 1.58, P = 0.03, respectively), high CD147 (HR= 2.13, $P < 0.005$), and moderate and high PPIA expression (HR= 2.74, $P < 0.005$ and HR= 2.01,$P < 0.005$, respectively) independently associated with poor-survival. Interestingly, removal of PPIA from the model demonstrated high PPIB expression significantly associated with poor-outcome (HR= 1.80, P = 0.01) (Supplemental Table S2) suggesting that PPIB prognostic effects rely on correlation with PPIA.

Multivariate analysis of samples stratified by *MYCN*amplification, age at diagnosis, tumor histology and the NTRK1-PTPN6-TP53 module, showed that in the presence of *MYCN*amplification, moderate ACE2 (HR= 1.51, P = 0.04), high CD147 (HR= 1.97, P = 0.01) and high and moderate PPIA expression (HR= 2.30,$P < 0.005$; HR= 1.85, P = 0.01) were significantly associated with poor-prognosis independent of *MYCN* amplification (Supplemental Table S3). Analysis in patients younger and older than 18 months of age showed moderate ACE2 (HR= 1.56, P = 0.03), high CD147 (HR= 1.99, P = 0.01) and high and moderate PPIA expression (HR= 2.27, P< 0.005, HR= 1.8, P = 0.01) significantly associated with poor-survival independent of age at diagnosis (Supplemental Table S4). Neuroblastomas are classified as differentiating or undifferentiated[5]. Adjustment by tumor histology showed high and moderate PPIA expression significant

associated with poor-survival independent of tumor histology (HR= 2.33,$P < 0.005$, HR= 1.65, $P = 0.04$) (Supplemental table S5).

These results were supported by Kaplan-Meier analysis which demonstrated significant separation in survival outcome between high, moderate and low expression of ACE2, CD147, PPIA or PPIB (Figure 1A-D). Patients with high ACE2 expression had a 59.4% probability of 5-year EFS with a median-survival-time of over 5-years, whereas low expression had a 73.3% probability of 5-year EFS with a median-survival-time of over 5-years. For CD147 high levels had a 38.1% probability of 5-year EFS with a median-survival-time of 1.83 years, whereas with low expression the probability increased to 81.6% with a median-survival-time of over 5-years. For PPIA and PPIB high expression had a 36.9% and 37.7%, respectively, survival probability with a median-survival-time of 1.8 and 2.23 years, whereas with low expression survival increased to 82.6% and 75.1%, with a median-survival-time of over 5-years.

Candidate gene expression was assessed in stratified groups with the independent t-test. *MYCN* amplified samples (Figure 1E-H) and those from patients older than 18 months of age (Supplemental Figure S1) were significantly associated with upregulation of ACE2, CD147, PPIA and PPIB. Similar results were obtained for GEO datasets (Supplemental Tables S6 and S7). In TARGET, results remained significant for PPIA and PPIB (Supplemental Tables S6 and S7). Unfavourable tumor histology was associated with upregulation of CD147, PPIA, and PPIB (supplemental Figure S2) in both GEO datasets (Supplemental Table S8). Significant association remained for PPIA and PPIB in TARGET (Supplemental Table S8).

2.2 ACE2, CD147, PPIA and PPIB association with NTRK1-PTPN6-TP53

To verify that samples expressing NTRK1-PTPN6-TP53 are indicative of good-prognosis[10], EFS was determined using Kaplan-Meier analysis (Supplemental Figure S3 A,B). It was estimated that patients with NTRK1-PTPN6-TP53 presence had an 81.7% probability of 5-year EFS with a median-survival-time of over 5-years, whereas in its absence the probability of 5-year EFS was 56.2% with a median-survival-time of 3.4 years. Similar results were obtained with an independent t-test (Supplemental Figure S3 C,D). Therefore, verifying association of the module with prolonged EFS[10].

Multivariate analysis of ACE2, CD147, PPIA and PPIB in samples expressing NTRK1-PTPN6-TP53 showed this module significantly associated with good-prognosis independent of ACE2, CD147, PPIA and PPIB expression (HR= 0.47, $P =<0.005$) (Supplemental Table S9). Similar results were obtained with an independent t-test (Supplemental Figure S4). In GEO datasets, NTRK1-PTPN6-TP53 was significantly associated with downregulation of ACE2, CD147, PPIA and PPIB and in TARGET for PPIA and PPIB (Supplemental Table S10).

Stratification by SCA was not undertaken as only TARGET had information regarding SCA in the form of 59 samples. This low sample number was not statistically powerful to carry out the analysis.

3. DISCUSSION

Together, these results demonstrate that the SARS-CoV-2 viral-entry ACE2, CD147, PPIA and PPIB genes, are upregulated and significantly associated with poor-prognosis neuroblastomas with MYCN amplification, unfavourable histology and in patients older than 18 months of age. Moreover, their expression is downregulated in tumors expressing NTRK1-PTPN6-TP53. This strongly suggests that poor-prognosis patients may be at a higher-risk of SARS-CoV-2 infection when compared to those expressing NTRK1-PTPN6-TP53.

These findings are supported by our preliminary identification of enriched-gene-clusters and enriched-pathways in prognosis-related clusters of neuroblastoma samples (using the TARGET dataset), with potential to interact with SARS-CoV-2 proteins. Results showed that in potential poor-prognosis clusters PI3K-AKT-mTOR signalling and CD147, PPIA, PPIB and NTRK1 could potentially be implicated in interactions with the SARS-CoV NSP3 protein. NSP3 is of relevance as SARS-CoV NSP3 shares 94% sequence homology with SARS-CoV-2 NSP3[26]. NSP3 has immuno-evasive properties by inhibiting interferon signalling and preventing attack from the host's immune system[27]. Interestingly, interferon signalling was prevalent in interactions

seen in poor-prognosis S-type and Mesenchymal neuroblastoma cell lineages. However, this needs further investigation due to the complex and heterogeneous nature of neuroblastoma.

These findings may have implications for patient care. CD147 participates in neuroblastoma tumor growth and metastasis[28]. Inhibition of CD147 in glioblastoma reduces tumor cell-invasion[29]. Meplazumab, a biological drug which blocks CD147 on the surface of Vero E6 cells and inhibits SARS-CoV-2 replication[30], could potentially treat SARS-CoV-2 infection and inhibit neuroblastoma growth.

Our results increase knowledge regarding neuroblastoma patient-risk to severe SARS-CoV-2 infection. Although studies have analysed SARS-CoV-2 infection rates in paediatric cancer patients[6], to date, none have specifically focused on neuroblastoma. Moreover, given that treatment of high-risk neuroblastoma needs further development, ACE2, CD147 and PPIA could be considered potential treatment targets.

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Figure Legend

FIGURE 1 Kaplan-Meier survival curves and Box plots for 492 neuroblastoma samples expressing ACE2, CD147, PPIA, and PPIB

For each gene analysed, samples were stratified into low, medium and high expression groups.

Kaplan-Meier curves were plotted for each group, and survival distributions curves were compared with the log rank test. Samples with high expression of ACE2, CD147, PPIA or PPIB had significantly decreased event free-survival (EFS) when compared to samples with low expression (Figure 1. A-D).

Samples were stratified into groups with and without *MYCN* amplification. Box plots were plotted for the two groups using EFS data. Independent t-tests and log rank tests were used to determine significance (Figure 1. E-H).

Conflict of Interest statement

The authors declare that they do not have competing financial interests or personal relationships that could influence the investigation described in this paper.

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FIGURE 1