Mutational Landscape of the Essential Autophagy Gene

BECN1 in Human Cancers

Saurabh V. Laddha¹, Shridar Ganesan¹, Chang S. Chan¹,², and Eileen White¹,³

Abstract

Evidence suggests that the catabolic process of macroautophagy (autophagy hereafter) can either suppress or promote cancer. The essential autophagy gene ATG6/BECN1 encoding the Beclin1 protein has been implicated as a haploinsufficient tumor suppressor in breast, ovarian, and prostate cancers. The proximity of BECN1 to the known breast and ovarian tumor suppressor breast cancer 1, early onset, BRCA1, on chromosome 17q21, has made this determination equivocal. Here, the mutational status of BECN1 was assessed in human tumor sequencing data from The Cancer Genome Atlas (TCGA) and other databases. Large deletions encompassing both BRCA1 and BECN1, and deletions of only BRCA1 but not BECN1, were found in breast and ovarian cancers, consistent with BRCA1 loss being a primary driver mutation in these cancers. Furthermore, there was no evidence for BECN1 mutation or loss in any other cancer, casting doubt on whether BECN1 is a tumor suppressor in most human cancers.

Implications: Contrary to previous reports, BECN1 is not significantly mutated in human cancer and not a tumor-suppressor gene, as originally thought.

Visual Overview: http://mcr.aacrjournals.org/content/early/2014/04/01/1541-7786.MCR-13-0614/F1.large.jpg. Mol Cancer Res; 12(4); 485–90. ©2014 AACR.

Introduction

Autophagy captures and degrades intracellular proteins and organelles in lysosomes to preserve protein and organelle quality and to recycle building blocks to sustain metabolism and survival in starvation (1, 2). Autophagy promotes the health, function, and survival of cells and tissues, and generally, the loss of autophagy is destructive. In mammals, autophagy deficiency is linked to tissue degeneration, chronic inflammation, susceptibility to metabolic stress, and premature lethality.

There is evidence that autophagy both promotes and suppresses cancer, however, this has not been rigorously assessed in humans (3). Monoallelic disruption of BECN1 on chromosome 17q21 has been reported in 40% to 75% of human breast, ovarian, and prostate tumors, suggesting that autophagy is a tumor-suppression mechanism (4–6). BECN1 allelic loss was also found in 9 out of 22 breast cancer cell lines by FISH analysis, although no coding or splice-site mutations were found (4). The small sample sizes and poorly matched comparisons of cell lines and normal tissues and the modest frequencies of loss of heterozygosity used for these investigations is, however, insufficient to support the claim that BECN1 is a haploinsufficient tumor suppressor.

BECN1 is located on chromosome 17q21 next to BRCA1, a known tumor-suppressor gene and whose loss is a driver of breast and ovarian cancer. BRCA1 is a critical regulator of DNA repair by homologous recombination and its loss causes DNA-repair defects and cancer predisposition (7). The close proximity of BECN1 and BRCA1 complicates the determination whether allelic loss of BECN1 is a driver or passenger mutation in breast and ovarian cancers. Furthermore, the mutational status of BECN1 in other cancers has not been rigorously assessed.

In support of the concept that autophagy is a tumor-suppression mechanism and that allelic loss of BECN1 promotes cancer, Beclin1+/- mice are prone to mammary hyperplasia, liver and lung carcinomas, and lymphomas (8, 9). However, mosaic whole-body knockout of the essential autophagy gene Atg5, or liver-specific knock out of the essential autophagy gene Atg7, produces only benign liver hepatomas and no other neoplasms (10). Thus, autophagy defects promote development of benign liver tumors in mice but may also block their progression. Autophagy-
independent functions of \textit{Becn1} may also contribute to the suppression of non-liver neoplasms or these events may be limited to genetically manipulated mice and not relevant to human cancers. The vast majority of germline mutations in \textit{BRCA1} are loss-of-function mutations (frameshift, indels, nonsense, or missense mutations), or focal deletions, not gross deletions in the \textit{BRCA1} locus at 17q21 that extend to encompass \textit{BECN1}. Thus \textit{Beclin1} \textsuperscript{−/−} mice do not reflect a human condition.

In contrast, autophagy promotes the survival of tumor cells in hypoxic tumor regions (11) as well as the growth, survival, and malignancy of RAS- and BRAF-driven cancers (3, 12–15). Autophagy promotes tumorogenesis by suppressing p53 activation and maintaining mitochondrial function essential for cellular metabolism and survival (16). Without autophagy, tumors accumulate defective mitochondria, have growth and metabolic defects, and progresses to a more benign fate. This is consistent with a large body of literature indicating that autophagy is required for survival in starvation and stress, functions that are conserved from yeast to mammals that are also important for growth of \textit{BRCA1} centric to \textit{BRCA1}.

Germline mutations in \textit{BRCA1}, \textit{BRCA2}, and \textit{PALB2} predispose to hereditary breast cancer and the three proteins function together to maintain genome stability by promoting faithful repair of double-strand breaks by homologous recombination (18). Mammary epithelial cell–specific knockout of \textit{Palb2} causes mammary tumorogenesis with long latency that is suppressed by allelic loss of \textit{Becn1}, suggesting that autophagy is tumor promoting (19). Deletion of \textit{Trp53} abrogates tumorogenesis impairment upon allelic loss of \textit{Becn1} in \textit{Palb2}-deficient mammary tumors, thus the combination of autophagy defect and loss of a critical DNA-repair mechanism augments the p53 antitumor response (19). Because loss of both \textit{Palb2} and autophagy promote DNA damage and p53 activation (18, 20, 21), this explains enhanced p53 activity and why autophagy suppresses the p53 response and mammary tumorogenesis.

The important unanswered question here is whether mutations in essential autophagy genes are found in human cancers using current genomic information, and if they are found, are they loss- or gain-of-function mutations? Note that recent assessment of oncogenes and tumor-suppressor genes assembled from the current human tumor sequencing data does not include any autophagy genes (22), but this was not examined specifically. To begin to resolve the potential conflicting role of autophagy in human cancer, we examined the publicly available human tumor sequencing and gene expression databases (including The Cancer Genome Atlas, TCGA) to determine the mutational and expression status of \textit{BECN1} in a broad array of human cancers. We first assessed \textit{BECN1} for single-nucleotide variations (SNV) and copy-number variations (CNV) in human breast, ovarian, and prostate cancer genome sequences. Because \textit{BECN1} is adjacent to \textit{BRCA1}, we specifically looked for deletions of \textit{BECN1} that do not encompass \textit{BRCA1}. We found enrichment for truncating mutations of \textit{BRCA1}, deletion of the chromosomal region that included \textit{BRCA1} only, and deletions affecting both \textit{BRCA1} and \textit{BECN1}, but not truncating mutations of \textit{BECN1} or deletions of only \textit{BECN1}. Analysis of all other cancers that lack \textit{BRCA1} deletion indicated no significant recurrence of SNVs or CNVs in \textit{BECN1}. Thus, \textit{BECN1} is not mutated or specifically deleted in human cancer, indicating that it is not a tumor-suppressor gene.

**Materials and Methods**

**CNVs**

To study the copy-number status of \textit{BECN1} and \textit{BRCA1} in different cancers, we downloaded more than 10,000 processed copy-number data from the TCGA portal...
(https://tcga-data.nci.nih.gov/tcga/). The TCGA Consortium collected tumor and matched normal samples from 24 different cancers from which to perform single-nucleotide polymorphism (SNP) and comparative genomic hybridization (CGH) microarray analysis on genomic DNA to find CNVs that are listed in Supplementary Table S1. The cancers for which we obtained CNV data include acute myeloid leukemia, bladder urothelial carcinoma, brain lower grade glioma, breast invasive carcinoma, cervical squamous cell carcinoma and endocervical adenocarcinoma, colon adenocarcinoma, glioblastoma multiforme, head and neck squamous cell carcinoma, kidney chromophobe, kidney renal clear cell carcinoma, kidney renal papillary cell carcinoma, liver hepatocellular carcinoma, lung adenocarcinoma, lung squamous cell carcinoma, lymphoid neoplasm diffuse large B-cell lymphoma, ovarian serous cyst adenocarcinoma, pancreatic adenocarcinoma, prostate adenocarcinoma, rectum adenocarcinoma, sarcoma, skin cutaneous melanoma, stomach adenocarcinoma, thyroid carcinoma, and uterine corpus endometrioid carcinoma. Tumor samples represent primary as well as metastatic tumors.

The TCGA consortium performed CNV calling and provided level 3 data for CNVs including segment mean values and number of markers for all CNVs detected. Briefly, the segment mean is the average of the log2 ratio of probes in the segment [log2 (observed intensity/reference intensity)] and represents the extent of copy number changes for that particular genomic segment, whereas the number of markers is the number of probes present in that segmental region. To extract a set of high confidence CNVs, we used a threshold of 0.2 in segment mean value for amplifications and −0.2 for deletions. We derived these thresholds by examining the distribution of segment mean values from tumor and normal samples. In addition, we require the number of markers spanning a CNV to be at least 10 to decrease false positives in calling CNVs. We tested the sensitivity of our method by finding the previously reported CNVs (amplifications in PIK3CA, EGFR, FOX1A, and HER2; deletions in MLL3, PTEN, RB1, and MAP2K4) in breast invasive cancer (23). We used the CNVs that pass these criteria for further analysis and identify all CNVs that overlap BECN1 or BRCA1.

We used the matched tumor and normal samples to determine the somatic CNVs. We identify CNVs as germline in the tumor if there was an overlapping CNV in the matched normal. The ratio of deletions to amplifications of somatic CNVs found across the genome provide a background ratio for comparison with the ratio found at a particular locus. If the ratio of deletion to amplifications is different at a locus than the genome average, then there may be selection for deletions or amplifications at that locus. We used the two-tailed Fisher exact test for determining statistical significance using the average number of deletions and duplications per sample for the background and the number of samples with deletion and amplifications for the locus.

**Somatic mutations**

The TCGA provides somatic mutations detected from whole genome and whole-exome sequencing from matched tumor and normal samples as level 2 data (Supplementary Table S2). We extracted the somatic mutations for BECN1 and BRCA1 and indicated their type as missense, nonsense, silent, splice site, and insertion or deletion resulting in frame shift or in-frame (Supplementary Tables S3 and S4).

**Gene expression**

TCGA RNA-seq level 3 data for all cancers, tumor and normal, were processed and normalized and we used the RSEM normalized values for gene expression. Fold change in BECN1 gene expression between tumor and normal tissue was calculated using median expression of tumors and normal. Significance of differential gene expression change in BECN1 is calculated using a two-tailed Wilcoxon test and Bonferroni corrected for multiple hypothesis testing.

**Results**

**CNVs in BECN1**

CNVs in BECN1 were assessed in the databases indicated in Table S1 from approximately 10,000 normal/tumor pairs. CNVs were classified into three groups defined by whether the CNV overlapped with BECN1 but not BRCA1, overlapped with BRCA1 but not BECN1, or overlapped with both BECN1 and BRCA1 (Table 1). Most of the CNVs detected are large and overlap both BECN1 and BRCA1. Each CNV was further identified as a deletion, amplification, or interrupting amplification if only a part of a gene was amplified (not included in the count for amplifications). As expected, breast and ovarian tumors were significantly enriched for having deletions in the locus containing both BECN1 and BRCA1 (Fig. 1 and Table 1).

Other tumor types that exhibited significant enrichment for deletions in both BECN1 and BRCA1 include kidney chromophobe and uterine corpus endometrioid carcinoma (Fig. 1 and Table 1). Tumor types found having enrichment for amplifications include bladder urothelial carcinoma, kidney renal papillary cell carcinoma, and lung adenocarcinoma. Closer examination found that the CNVs in kidney chromophobe and kidney renal papillary cell carcinoma are whole-chromosome deletions and amplifications, respectively, which are consistent with known loss and gain of chromosome 17 for these two types of tumors (24).

CNVs that overlap BRCA1 but not BECN1 were enriched for deletions in breast and ovarian tumors, whereas CNVs that overlap BECN1 but not BRCA1 were not enriched for deletions in any tumor (Table 1). These results are consistent with the loss of BRCA1 being the driver mutation in breast and ovarian tumors. No significant CNVs in BECN1 were detected in any other cancers (Table 1). Loss of chromosome 17q21 and BRCA1 has been reported in prostate cancer only very infrequently (0.45%; ref. 25). For prostate adenocarcinoma, we found 9 deletions (covering both BECN1 and BRCA1) and no amplifications (Table 1). The P value for enrichment of deletions is 0.024, however, after correcting for multiple testing of the whole chromosome.
Table 1. Enrichment for deletions and amplifications for BECN1 and BRCA1 in cancers

<table>
<thead>
<tr>
<th>Cancer</th>
<th>Number of tumor/normal pairs</th>
<th>Average CNA per tumor BECN1 alone</th>
<th>BRCA1 alone</th>
<th>Both BECN1 and BRCA1</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Deletion</td>
<td>Amplification</td>
<td>Deletion</td>
</tr>
<tr>
<td>Acute myeloid leukemia</td>
<td>196</td>
<td>146</td>
<td>146</td>
<td>1</td>
</tr>
<tr>
<td>Bladder urothelial carcinoma</td>
<td>184</td>
<td>99</td>
<td>106</td>
<td>0</td>
</tr>
<tr>
<td>Brain lower grade glioma</td>
<td>269</td>
<td>34</td>
<td>32</td>
<td>1</td>
</tr>
<tr>
<td>Breast invasive carcinoma</td>
<td>999</td>
<td>85</td>
<td>111</td>
<td>3</td>
</tr>
<tr>
<td>Cervical squamous cell carcinoma and endocervical adenocarcinoma</td>
<td>155</td>
<td>52</td>
<td>61</td>
<td>1</td>
</tr>
<tr>
<td>Colon adenocarcinoma</td>
<td>460</td>
<td>70</td>
<td>71</td>
<td>3</td>
</tr>
<tr>
<td>Glioblastoma multiforme 1</td>
<td>434</td>
<td>29</td>
<td>25</td>
<td>1</td>
</tr>
<tr>
<td>Glioblastoma multiforme 2</td>
<td>237</td>
<td>30</td>
<td>27</td>
<td>2</td>
</tr>
<tr>
<td>Glioblastoma multiforme 3</td>
<td>170</td>
<td>35</td>
<td>29</td>
<td>0</td>
</tr>
<tr>
<td>Glioblastoma multiforme 4</td>
<td>534</td>
<td>106</td>
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<td>Head and neck squamous cell carcinoma</td>
<td>390</td>
<td>49</td>
<td>55</td>
<td>0</td>
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<td>Kidney chromophobe</td>
<td>66</td>
<td>97</td>
<td>75</td>
<td>1</td>
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<tr>
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<td>35</td>
<td>44</td>
<td>0</td>
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<tr>
<td>Kidney renal papillary cell carcinoma</td>
<td>168</td>
<td>30</td>
<td>49</td>
<td>0</td>
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<tr>
<td>Liver hepatocellular carcinoma</td>
<td>136</td>
<td>52</td>
<td>88</td>
<td>0</td>
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<tr>
<td>Lung adenocarcinoma</td>
<td>505</td>
<td>50</td>
<td>66</td>
<td>0</td>
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<tr>
<td>Lung squamous cell carcinoma 1</td>
<td>117</td>
<td>51</td>
<td>50</td>
<td>1</td>
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<td>Lung squamous cell carcinoma 2</td>
<td>505</td>
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<td>2</td>
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<td>Lymphoid neoplasm diffuse large</td>
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<td>49</td>
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<tr>
<td>B-cell lymphoma</td>
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<td>Ovarian serous cystadenocarcinoma 1</td>
<td>587</td>
<td>105</td>
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<tr>
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<td>363</td>
<td>16</td>
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<td>Pancreatic adenocarcinoma</td>
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<td>29</td>
<td>30</td>
<td>0</td>
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<tr>
<td>Prostate adenocarcinoma</td>
<td>195</td>
<td>46</td>
<td>29</td>
<td>1</td>
</tr>
<tr>
<td>Rectum adenocarcinoma</td>
<td>167</td>
<td>81</td>
<td>83</td>
<td>0</td>
</tr>
<tr>
<td>Sarcoma</td>
<td>84</td>
<td>71</td>
<td>122</td>
<td>0</td>
</tr>
<tr>
<td>Skin cutaneous melanoma</td>
<td>338</td>
<td>54</td>
<td>78</td>
<td>0</td>
</tr>
<tr>
<td>Stomach adenocarcinoma</td>
<td>306</td>
<td>44</td>
<td>59</td>
<td>1</td>
</tr>
<tr>
<td>Thyroid carcinoma</td>
<td>505</td>
<td>15</td>
<td>18</td>
<td>0</td>
</tr>
<tr>
<td>Uterine corpus endometrioid carcinoma</td>
<td>505</td>
<td>62</td>
<td>91</td>
<td>3</td>
</tr>
</tbody>
</table>

NOTE: Only statistically significant enrichments have their Bonferroni corrected P values shown. CNA, copy number alteration.
for multiple hypothesis testing, it is not significant. Prostate adenocarcinoma is a heterogeneous disease and the fraction of this disease where loss of 17q21 is a driver mutation is small compared with breast or ovarian cancer. It is clear, however, that in contrast with previous reports, BECN1 deletions do not significantly occur in the absence of BRCA1 deletion.

Somatic mutations

There are 169 and 32 (ratio of 5.28) mutations found in BRCA1 and BECN1, respectively, across all tumor samples (6,632) and the numbers are 137 and 31 (ratio of 4.42) if we exclude breast and ovarian tumors in which BRCA1 is known to be a tumor suppressor (Supplementary Table S2). The difference in mutation number is mostly explained by the size of the coding region of the two genes (ratio of protein coding length of BRCA1 to BECN1 is 4.14).

None of the mutations found in BECN1 were nonsense or splice-site mutations (Supplementary Table S3) with the potential to alter function and that are frequently found tumor-suppressor mutations. If we restrict analysis to breast and ovarian cancer, there is only one mutation found in BECN1 and it is a missense mutation in an ovarian tumor. In contrast, there are 32 mutations in BRCA1 of which 23 are nonsense, splice site, or frameshift mutations, all of which lead to truncation of BRCA1 (Supplementary Table S4).

Across all cancer data from TCGA, there are 30 missense, 0 nonsense, 0 splice site, and 11 silent mutations for BECN1 and 135 missense, 20 nonsense, 12 splice site, and 39 silent mutations for BRCA1. To find statistical enrichment of missense, nonsense, or splice-site mutations compared with silent mutations, we use as null model the aggregate of mutations across all samples in breast cancer (778 tumors) yielding 31,861 missense, 2,339 nonsense, 1,075 splice site, and 11,677 silent mutations. Because the vast majority of mutations detected in tumors are passenger mutations with little or no selective advantage to the tumors, the ratio of missense to silent mutations (2.73), nonsense to silent mutations (0.20), and splice site to silent mutations (0.09) are good approximations for little or no selection of missense, nonsense, or splice site over silent mutations. Indeed, these ratios are very similar when looking at other cancer types from TCGA. There is statistically significant increase in ratio of nonsense to silent and splice site to silent mutations for BRCA1 (2.6 and 3.4 fold increase with P value of 0.0008 and 0.0003 using two-tailed χ² test with Yate’s correction). There is no significant increase in missense over silent mutations for BRCA1 and BECN1, and no increase in nonsense and splice site over silent mutations in BECN1. The proportion of missense, nonsense, and splice-site mutations for BECN1 is statistically consistent with the occurrence of passenger mutations.

Gene-expression changes

The differential expression of BECN1 between tumor and normal tissue for 17 cancer types from TCGA show no significant fold-change greater than 2 (Supplementary Table S5). The greatest decrease in expression of BECN1 occurs in kidney chromophobe in which the fold-change of tumor to normal is 0.65, which is consistent with loss of chromosome 17 being common in this cancer.

Discussion

Using the genomic information collected on a broad array of human cancers, we assessed the mutational status of the essential autophagy gene BECN1. Despite reports indicating allelic loss of BECN1 in some human cancers, this seems to be explained solely by the proximity of BECN1 to BRCA1. We find no evidence of mutation or focal loss of BECN1 from the analysis of currently available cancer genomic information. Monoallelic loss of the chromosome 17q21 region that encompasses both BECN1 and BRCA1 is found in both breast and ovarian cancer. However, as the region is large, this finding does not support a role for BECN1 as the driver. Furthermore, there is no finding of nonsense or splice-site mutations in BECN1 in any other cancers.

Germline missense mutations in BRCA1 followed by somatic deletion of the remaining allele in tumors are responsible for inherited cancers. In those cancers, the majority of the deletions are large and take out both BRCA1 and BECN1 and a hundred others. While focal deletions and somatic, predicted loss-of-function mutations (missense, nonsense, frameshift, and splice-site mutations) are found in BRCA1, they are not found in BECN1. Furthermore, there are no significant germline mutation or allelic loss of BECN1 in patients with breast and ovarian cancer, nor are there inactivating mutations in the absence of BRCA1 mutation or loss. This is in agreement with BRCA1 deficiency being a driver mutation in breast and ovarian cancer. Indeed, allelic loss of Beclin1 suppresses, rather than promotes, mammary tumorigenesis mediated by Palb2 deficiency (19). As PALB2 is a regulator of BRCA1 and BRCA2 and a known tumor suppressor (18), this suggests that Beclin1 suppresses tumorigenesis of homologous recombination-deficient cancers rather than promoting it.

One interesting tumor type where autophagy may promote tumor progression not included in the analysis here is hepatomas. Mice with allelic loss of Beclin1, or biallelic deletion of Atg5 or Atg7 in liver are prone to liver tumors. Autophagy deficiency may promote initiation of benign liver tumors by inducing chronic tissue damage, but also autophagy may be needed for progression to more aggressive disease. Indeed, deletion of Atg7 diverts progression of lung adenocarcinomas to benign oncocytomas (13, 14). It will be of interest to examine the mutational status of autophagy genes in human hepatomas and oncocytomas once the sequencing data become available. This will test if autophagy defects both promote the genesis of hepatomas while they limit tumor progression to benign disease (hepatomas and oncocytomas).

Disclosure of Potential Conflicts of Interest

E. White is a consultant/advisory board member of Forma Therapeutics. No potential conflicts of interest were disclosed by the other authors.
Authors’ Contributions

Conception and design: S. Ganesan, C.S. Chan, E. White
Development of methodology: S. V. Laddha, C.S. Chan
Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.): S. V. Laddha, C.S. Chan
Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): S. V. Laddha, C.S. Chan
Writing, review, and/or revision of the manuscript: S. V. Laddha, S. Ganesan, C.S. Chan, E. White
Administrative, technical, or material support (i.e., reporting or organizing data, constructing databases): S. V. Laddha
Study supervision: C.S. Chan

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References

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