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Original Article

Up-regulated iNOS/NOS2 is associated with the poor prognosis of colorectal cancer: an integrated bioinformatics analysis

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Abstract: Objective: To investigate the nitric oxide synthase 2 (iNOS/NOS2) gene expression in colorectal carcinoma (CRC) and its association with the patients' prognosis. Methods: iNOS/NOS2 mRNA expression level between cancer and normal tissue of colorectal cancer subjects were compared through the Cancer Genome Atlas (TCGA). Protein-protein interaction (PPI) network was constructed by the STRING database of iNOS/NOS2 gene. The hub genes involved in the network was identified by cytoscape. Biological function, pathway of iNOS/NOS2 and hub genes were enriched through GO and KEGG analysis. Correlation between OS, DFS and iNOS/NOS2 expression was analyzed by cox proportional hazard regression analysis in the TCGA database. Results: iNOS/NOS2 expression of cancer tissue was significantly higher compared to corresponding normal tissue of CRC subjects ($P < 0.05$). 51 nodes and 366 edges with the average node degree of 14.4 was constructed which indicated that the PPI enrichment was statistically significant ($P < 1.0e-16$). And ten hub genes (NOS2, RPS27A, UBC, UBB, EHHADH, ACOX1, AGXT, CAT, PIPOX and IDH1) were identified by the cytoscape. The ten hub genes were mainly enriched in the protein targeting to peroxisome, cellular catabolic process, carboxylic acid catabolic process, coenzyme binding, oxidoreductase activity, signaling receptor binding, peroxisomal matrix, host cell, cytosol and ect for gene ontology. And peroxisome, carbon metabolism, PPAR signaling pathway were enriched in the KEGG pathway analysis. Kaplan-Meier plot showed that high expression of iNOS/NOS2 was associated with the poor overall survival (HR=0.6, P=0.019) and disease-free survival (HR=0.65, P=0.049) of colorectal cancer patients. Conclusion: iNOS/NOS2 was up-regulated in CRC and associated with poor prognosis of CRC which maybe a potential biomarker for this disease.

Keywords: Colorectal cancer, iNOS/NOS2, survival, bioinformatics

Introduction

Epidemiological data indicated that the incidence of CRC ranked fourth among all the malignant carcinomas [1]. It was reported that there were 143460 new cases of colorectal cancer in the United States and 51690 deaths in the year 2012 [2]. Although CRC was one of the leading causes of malignant carcinoma associated death worldwide, the pathogenesis of CRC is not completely clear [3, 4]. In recent years, with the progression of molecular biology technology, the mechanism of malignant biological behavior of colorectal was known as a multi-gene and multi-step molecular biological regulation process, in which a variety of genes were involved [5-7].

iNOS was up-regulated in some malignant carcinomas and correlated with cell proliferation and migration [8-10]. In our present manuscript, iNOS/NOS2 expression in CRC and its association with the patients' survival was evaluated by integrated bioinformatics analysis in order to investigate more information of iNOS/NOS2 in colorectal carcinoma.

Material and methods

iNOS/NOS2 expression analysis

iNOS/NOS2 mRNA in tumor and corresponding normal tissue were analyzed in TCGA database through the Gene Expression Profiling Interactive Analysis (GEPIA) [11] online data min-

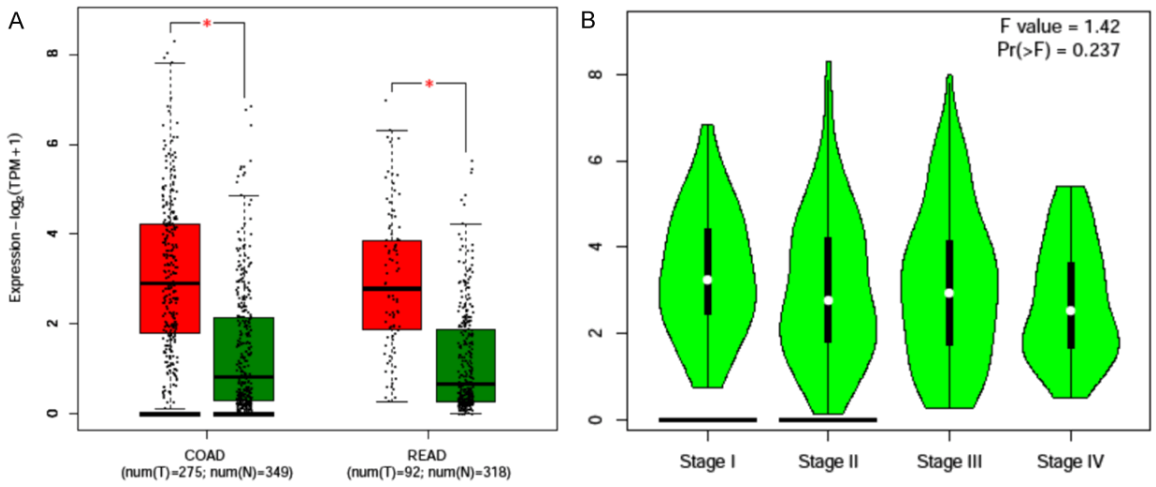

Figure 1. iNOS/NOS2 mRNA expression in CRC patients (A: iNOS/NOS2 mRNA expression comparison between cancer and corresponding normal tissue; B: Correlation between iNOS/NOS2 mRNA level and clinical stage of colorectal patients).

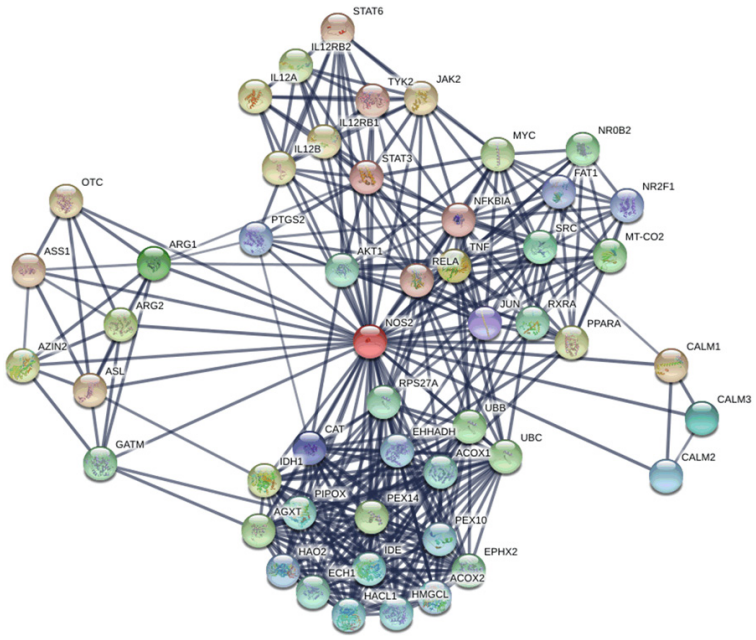

Figure 2. PPI network of iNOS/NOS2 and relevant genes.

ing web site (http://gepia.cancer-pku.cn/index.html).

PPI network and hub genes analysis

The PPI network of iNOS/NOS2 and related genes were established through the Search Tool for The Retrieval of Interacting Genes (STRING) under the conditions of max number of interactors < 50 and minimum required interaction score of 0.4. 10 hub genes was further determined through cytoscape [12, 13].

GO and KEGG analysis

The biological effects and possible pathways of iNOS/NOS2 and ten hub genes were enriched by gene ontology and KEGG pathway analysis. Biological process (BP), cellular component (CC) and molecular function (MF) were enriched for GO. Bubble char was used to demonstrated the biological function and pathway enrichment [14].

Prognosis evaluation

According the median iNOS/NOS2 mRNA level, CRC subjects were divided into high and low expression group. Kaplan-Meier curve was drawn according to iNOS/NOS2 high and low expression group and compared through log-rank test. The overall survival (OS) and disease free survival (DFS) was compared between the iNOS/NOS2 high and low expression group.

Results

iNOS/NOS2 mRNA expression

iNOS/NOS2 mRNA expression of cancer tissue was significantly higher compared to corresp-

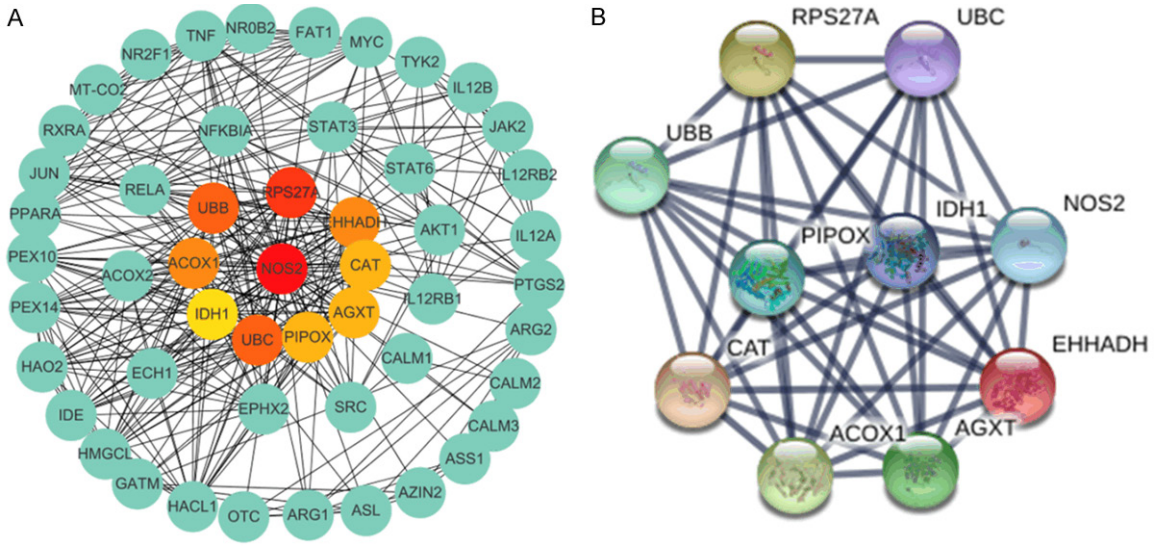

Figure 3. Hub genes identification of iNOS/NOS2 and relevant genes of the PPI network (A: 10 hub genes were identified; B: PPI network of the 10 hub genes).

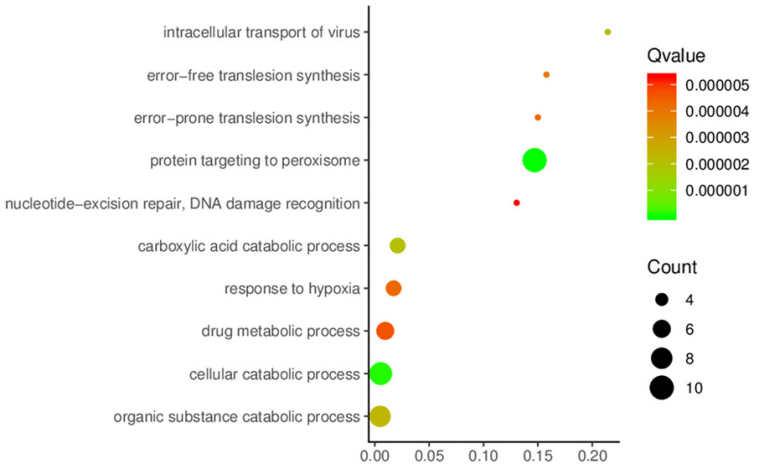

Figure 4. Gene ontology analysis of iNOS/NOS2 in the aspect of biological process.

onding normal tissue of CRC subjects ($P < 0.05$) (**Figure 1A**). But iNOS/NOS2 mRNA expression was not correlated with the clinical stages of colorectal patients ($P > 0.05$) (**Figure 1B**).

PPI network and hub genes

51 nodes and 366 edges with the average node degree of 14.4 were constructed which indicated that the PPI enrichment was statistically significant ($P < 1.0e-16$) (**Figure 2**). And ten hub genes (NOS2, RPS27A, UBC, UBB, EHHADH, ACOX1, AGXT, CAT, PIPOX and IDH1) were determined through cytoscape (**Figure 3**).

GO and KEGG analysis

The ten hub genes were enriched in the protein targeting to peroxisome, cellular catabolic process, carboxylic acid catabolic process and ect for biological process (**Figure 4**). For molecular function, the ten hub genes were enriched in coenzyme binding, oxidoreductase activity, signaling receptor binding and ect (**Figure 5**). And in the aspects of cellular component, the ten hub genes were enriched in peroxisomal matrix, host cell, cytosol and etc (**Figure 6**). And Peroxisome, Carbon metabolism, PPAR signaling pathway and ect were enriched in the KEGG pathway (**Figure 7**).

Survival analysis

Kaplan-Meier plot showed that high expression of iNOS/NOS2 was related to the poor OS (HR=0.6, P=0.019) and DFS (HR=0.65, P=0.049) of CRC patients (**Figure 8**).

Discussion

iNOS was reported to be up-regulated in various malignant carcinoma such as NSCLC, gastrointestinal cancer, and breast carcinoma [10,

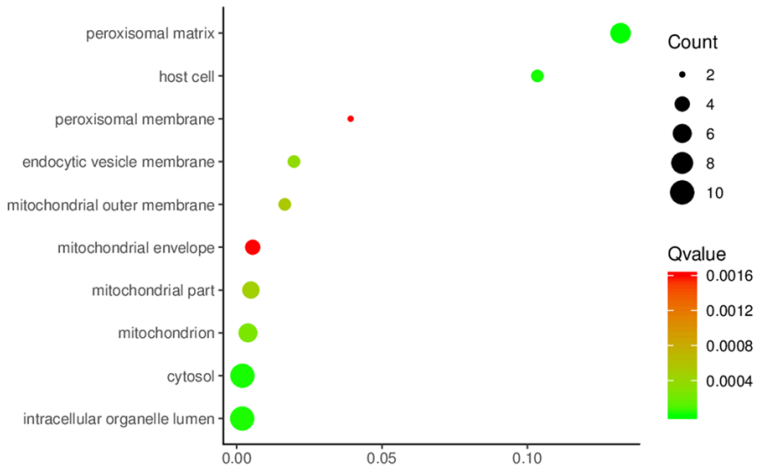

Figure 5. Gene ontology analysis of iNOS/NOS2 in the aspect of cellular component.

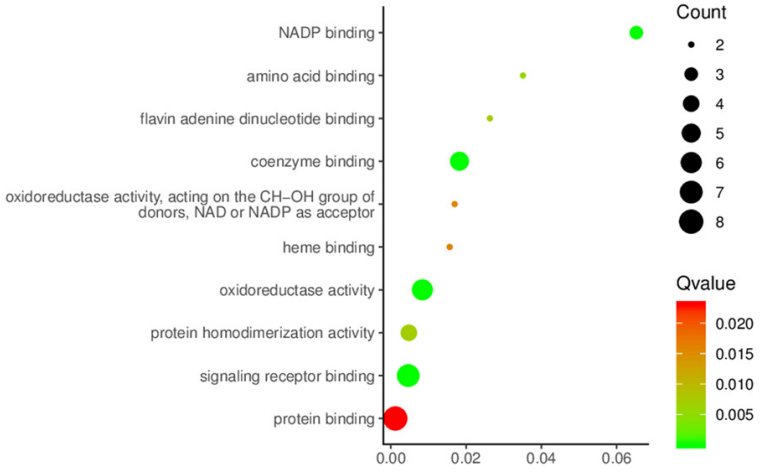

Figure 6. Gene ontology analysis of iNOS/NOS2 in the aspect of molecular function.

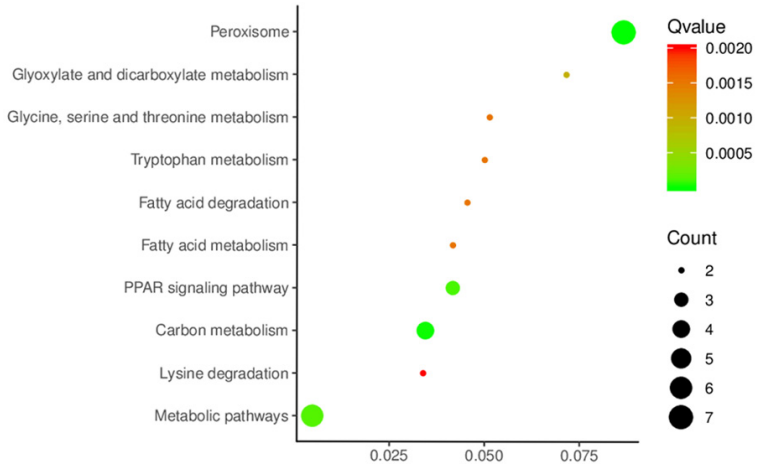

Figure 7. KEGG pathway analysis of iNOS/NOS2 and relevant genes.

15-17]. NO is a bioactive factor, which is produced by L-arginine catalyzed by nitric oxide synthase (NOS) in vivo. There are three main types of NOS [18]: eNOS, which mainly exists in vascular endothelial cells, neuronal nitric oxide synthase (nNOS) in nerve cells, and iNOS which is expressed in many kinds of cells in pathological conditions. Studies have shown that iNOS can be activated by inflammatory cytokines or endotoxins, and then synthesize large amounts of NO [19, 20]. The level of iNOS can reach 1000 times that of eNOS and nNOS. Excess NO can nitride and modify key proteins, change signaling pathways, and lead to many diseases, such as septic shock, cancer, asthma, inflammatory bowel disease, etc [21, 22]. Concentration-dependent mechanism of NO dual effects on tumorigenesis or anti-tumorigenesis is generally believed that sustained low concentration of NO can promote the growth of tumor cells, participate in tumor angiogenesis or inhibit the apoptosis of tumor cells. The possible mechanism of high concentration of NO in anti-tumor is to interfere with the energy metabolism of tumor cells, generate oxygen free radicals to produce cytotoxicity, and mediate macrophage activation. The expression of iNOS in normal tissues is extremely low. The role of NO in colon tumorigenesis can be evaluated by investigating the expression of iNOS. Lv et al [23] evaluated the correlation between clinicopathological features and iNOS expression in CRC patients. The authors found that iNOS protein positive expression was associated with lymph node metastasis and

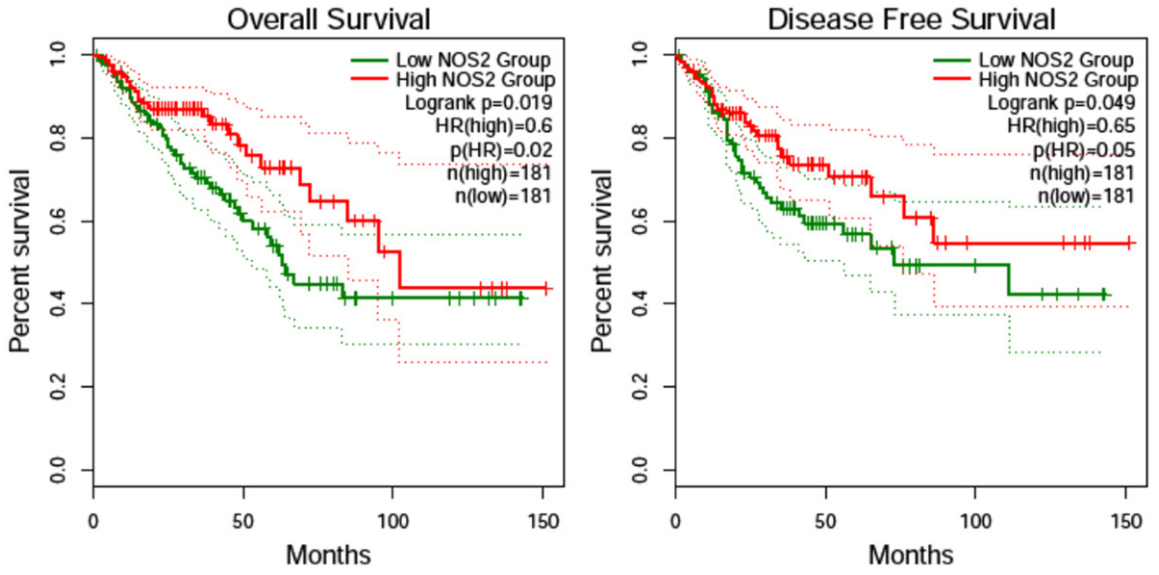

Figure 8. Kaplan-Meier plot showed that high expression of iNOS/NOS2 was associated with the poor overall survival and disease-free survival of CRC patients.

clinical stage ($P < 0.05$). This indicated that iNOS can be used as prognostic indicators of CRC.

In our study, we investigated the iNOS/NOS2 expression in CRC and correlation with the patients' prognosis. We found that iNOS/NOS2 was up-regulated in cancer tissue compared to normal tissue in CRC patients which was in accordance with previously relevant studies [23, 24]. Ten hub genes (NOS2, RPS27A, UBC, UBB, EHHADH, ACOX1, AGXT, CAT, PIPOX and IDH1) were identified which were mainly enriched in the aspects of cancer cell proliferation, migration or invasion. Survival analysis indicated that high expression of iNOS/NOS2 was a poor indicator for CRC patients. Although our findings indicated that high expression of iNOS/NOS2 was correlated with poor prognosis of CRC which maybe a potential biomarker for this disease, its molecular mechanism in oncogenesis needs further investigation in *vivo* and in *vitro*.

Disclosure of conflict of interest

None.

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