Inhibiting tryptophan metabolism enhances interferon therapy in kidney cancer

Supplementary Material

Table S1. PCR primers used for quantitative PCR.

Accession Number	Gene	Forward and Reverse Primers (5'-3')	Tm[1] (^{o}C)	E[2] (%)
NM_005651	h*TDO2*	GCACTTCAGGGAGCATTGAT TCACTCACAGTTGATCGCAG	64	103
	h*IDO1*[3]	GGTCATGGAGATGTCCGTAA ACCAATAGAGAGACCAGGAAGAA	62	97
NM_019911	m*Tdo2*	ATGGCTGGAAAGAACACCTG CATCAAACAAGCAGAGCAGC	63	79
	m*Ido1*[4]	GTACATCACCATGGCGTATG CGAGGAAGAAGCCCTTGTC	60	88
NM_001289726	m*Gapdh*	TGATGGGTGTGAACCACGAG AAGTCGCAGGAGACAACCTG	63	53
	Rn18S[5]	ACGGCTACCACATCCAAGGA CCAATTACAGGGCCTCGAAA	60	90
	PPIA[6]	ACCGCCGAGGAAAACCGTGTA TGCTGTCTTTGGGACCTTGTCTGC	64	95
	RPS13[6]	TCGGCTTTACCCTATCGACGCAG ACGTACTTGTGCAACACCATGTGA	64	101

[1]Annealing and extension temperature.

[2]Primer pair efficiency (E).

[3]Reisenberg *et al*. [1]

[4]Uyttenhove *et al*. [2]

[5]Rowson-Hodel *et al*. [3]

[6]Dupasquier *et al.* [4]

qPCR Methods

Total RNA was extracted from tissue or cells using Trizol (Invitrogen) and its integrity confirmed by gel electrophoresis. Primers for qPCR (Table S1) were designed using Primer Blast (http://www.ncbi.nlm.nih.gov/tools/primer-blast/) to be in different exons and not amplify non-specific cDNA or gDNA. This was confirmed by performing

PCR on reverse transcription negative control reactions performed in the absence of reverse transcriptase. The tryptophan-2,3-dioxygenase (*TDO2*) and mouse glyceraldehyde-3-phosphate dehydrogenase (*Gapdh)* PCR products were confirmed by sequencing. Reference genes for mouse samples were 18S ribosomal RNA (*Rn18S*) and *Gapdh* (Table S1). Reference genes for human samples were cyclophilin A (*PPIA*) and ribosomal protein S13 (*RPS13*) as described by Dupasquier *et al*., [4; Table S1]. Total RNA (0.5 μg) was reverse transcribed in 20 μl using MultiScribe reverse transcriptase (Thermo Fisher Scientific; 50U). The resulting cDNA was diluted (1:4 or 1:50) before 1 μl was analyzed using SYBR green PCR master mix (Applied Biosystems, Foster City, CA) with 0.25 μM primers on a ViiA™ 7 Real-Time PCR System (Applied Biosystems). Cycling conditions were 50°C for 2 min, 95°C for 10 min then 40 cycles of 95°C for 15 s and 1 min at 60, 62, 63 or 64^{o}C (Table S1). Each qPCR run included a no-template control containing all reagents except cDNA. Standard curves were prepared using 6 to 7 five-fold serial dilutions of either mouse or human liver cDNA. One standard curve was used for all qPCR plates within an experiment. All standard curves had a linear regression coefficient of determination of at least 99.4%. The mRNA or rRNA levels in each mouse sample were calculated from Ct values using a standard curve. The relative mRNA levels in each human sample were calculated from Ct values using the delta-delta Ct method relative to an average of the two housekeeping Ct values.

Table S2. Immunohistochemistry staining score of RCC and adjacent normal kidney tissue.

Antibody	IDO1[3]	IDO1	IDO1	CD68[4]
Tissue	Endothelial cells	Neoplastic cells	Interstitial cells	Interstitial cells
Kidney Neg[1]	0	0	0	n/a
Kidney-1	0	0	0	0.5
Kidney-2	0	0	0	0.5
Kidney-3	0	0	0	1
Grade 2[2] Neg	0	0	0	0
Grade 2-1	1	0	1	2.5
Grade 2-2	3	1	1	3
Grade 2-3	2	1	1	2
Grade 3 Neg	0	0	0	0
Grade 3-1	2	0	1	4
Grade 3-2	2	0	1	5
Grade 3-3	3	0	1	2.5

[1]Neg=secondary antibody only

[2]Grade of renal cell carcinoma (RCC)

[3]Score for IDO1: 0=no staining, 1=1-10%, 2=10-20%, 3=20-40% staining, respectively

[4]Score for CD68: 0=no staining, 1=1-10%, 2=10-20%, 3=20-30%, 4=30-40%, 5=40-50% positive interstitial cells

REFERENCES

1. Riesenberg R, Weiler C, Spring O, Eder M, Buchner A, Popp T et al. Expression of indoleamine 2,3-dioxygenase in tumor endothelial cells correlates with long-term survival of patients with renal cell carcinoma. Clin Cancer Res. 2007; 13(23):6993-7002.
2. Uyttenhove C, Pilotte L, Theate I, Stroobant V, Colau D, Parmentier N et al. Evidence for a tumoral immune resistance mechanism based on tryptophan degradation by indoleamine 2,3-dioxygenase. Nat Med. 2003; 9(10):1269-74.
3. Rowson-Hodel AR, Manjarin R, Trott JF, Cardiff RD, Borowsky AD, Hovey RC. Neoplastic transformation of porcine mammary epithelial cells in vitro and tumor formation in vivo. BMC Cancer. 2015; 15:562.
4. Dupasquier S, Delmarcelle AS, Marbaix E, Cosyns JP, Courtoy PJ, Pierreux CE. Validation of housekeeping gene and impact on normalized gene expression in clear cell renal cell carcinoma: critical reassessment of YBX3/ZONAB/CSDA expression. BMC Mol Biol. 2014; 15:9.