

Supplements

Neuropilin-2 and its transcripts correlate with clinical outcome in bladder cancer

Table S1: Primers and probes used in this study

Gene	Forward Primer Sequence (5' → 3')	Reverse Primer Sequence (5' → 3')	Probe Sequence (5' label → 3' label)
<i>CALM2</i>	GAGCGAGCTGAGTGGTTGTG	AGTCAGTTGGTCAGCCATGCT	VIC-TCGCGTCTCGGAAACCG GTAGC-BHQ1
<i>NRP1</i>	CCCCAAACCACTGATAACTCG	GTCCAGACACCATACCCAACAT	FAM-CGAATCAAGCCTGCAACT TGGGAACTGG-BHQ1
<i>NRP2</i>	GGGGGACTGCAAGTACGATT	AGAGGATCCCCGTCGATGAA	FAM-CTGGACATCTGGGATGGCA TTCCACATG-BHQ1
<i>NRP2a</i>	GGAGAACTGCATGGAACCCA	AATTGCTCCAGTCCACCTCG	FAM-CATCATCAATTTTCATCTTC ATATCCTTCTCTCTCATG- BHQ1
<i>NRP2b</i>	ATGTCCCACTGGAGAACTGC	ATACCAGTAGGCTGGGATGGG	FAM-GCATGGGCACCGTGTCC ACTGTGG-BHQ1
<i>PDGFC</i>	TCCTGGTTAAACGCTGTGGT	GACACCGGTCTTTGGTCTCA	FAM-CTGTGCCTGTTGTCTCC ACAATTGCAATGAATG- BHQ1
<i>PDGFD</i>	TGGAAGATTTCCAACCCGCA	GCAATCAGAGTGGGATCCGT	FAM-CCAAGTGGGAATCTGTCA CAAGCTCTATTTTC-BHQ1

Table S2: NRP2 transcript variant expression in TCGA cohort

Variant (n)	Median (IQR)
NRP2A (360)	8.83 (7.55-9.87)
NRP2 A22 (120)	5.10 (0-7.38)
NRP2 A17 (285)	8.39 (6.64-9.47)
NRP2 A0 (211)	4.46 (0-6.31)
NRP2B (357)	5.61 (3.99-6.94)
NRP2 B5 (70)	0 (0-4.18)
NRP2 B0 (335)	5.34 (3.67-6.77)
NRP2 S9 (243)	1.84 (0-3.06)

Abbreviations: IQR, interquartile range

Table S3: Ratio of NRP2A/NRP2B transcript variants in TCGA and the Mannheim cohort

NRP2A/NRP2B	>1	≤1
TCGA cohort (n, %)	360 (100)	0 (0)
Mannheim cohort (n, %)	95 (93.1)	7 (6.9)

Table S4: Correlation of NRPs and indicated ligands in TCGA cohort

Marker		NRP2	NRP2A	NRP2B	PDGFC	PDGFD
NRP1	ρ	0.6391	0.6451	0.6417	0.5376	0.1461
	P value	<0.0001	<0.0001	<0.0001	<0.0001	0.0079
NRP2	ρ		0.9977	0.9346	0.7396	0.1243
	P value		<0.0001	<0.0001	<0.0001	0.0241
NRP2A	ρ			0.9362	0.7394	0.1239
	P value			<0.0001	<0.0001	0.0246
NRP2B	ρ				0.6773	0.0549
	P value				<0.0001	0.3209
PDGFC	ρ					0.2507
	P value					<0.0001
PDGFD	ρ					
	P value					

Abbreviations: ρ, Spearman correlation

Table S5: Correlation of NRPs and indicated ligands in the Mannheim cohort

Marker		NRP2	NRP2A	NRP2B	PDGFC	PDGFD
NRP1	ρ	0.7272	0.6985	0.5816	0.7624	0.6764
	P value	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
NRP2	ρ		0.8413	0.6684	0.6869	0.6730
	P value		<0.0001	<0.0001	<0.0001	<0.0001
NRP2A	ρ			0.5360	0.6659	0.6268
	P value			<0.0001	<0.0001	<0.0001
NRP2B	ρ				0.5832	0.5525
	P value				<0.0001	<0.001
PDGFC	ρ					0.5226
	P value					<0.0001

Abbreviations: ρ, Spearman correlation

Table S6: Univariable and multivariable Cox regression analyses to test the effect of different parameters on disease-free survival in the TCGA cohort

Variable	Univariable analysis			Multivariable analysis		
	HR	95% CI	P value	HR	95% CI	P value
Age (high/low)	1.24	0.86-1.78	0.2432			
Gender (male/female)	0.91	0.62-1.37	0.6507			
T stage (T3/4 / T2)	2.59	1.69-4.11	<0.0001	2.05	1.13-3.98	0.0170
N stage (pos/neg)	2.42	1.67-3.53	<0.0001	1.48	0.89-2.78	0.1292
LVI (yes/no)	2.31	1.50-3.63	0.0001	1.53	0.89-2.64	0.1265
NRP1 (high/low)	1.61	1.04-2.41	0.0327	1.35	0.77-2.28	0.2876
NRP2 (high/low)	2.29	1.59-3.27	<0.0001	2.41	0.38-9.71	0.3101
NRP2A (high/low)	2.23	1.55-3.19	<0.0001	0.67	0.17-4.15	0.6163
NRP2B (high/low)	2.23	1.54-3.20	<0.0001	1.32	0.71-2.43	0.3788
PDGFC (high/low)	1.66	1.15-2.38	0.0072	0.90	0.49-1.64	0.7342
PDGFD (high/low)	1.48	1.02-2.13	0.0389	1.67	1.02-2.63	0.0332

Abbreviations: HR, hazard ratio; CI, confidence interval; LVI, lymphovascular invasion

Table S7: Associations of ABSOLUTE and ESTIMATE scores with T stage

	ABSOLUTE	ESTIMATE
Variable (n)	Median (IQR)	Median (IQR)
T stage		
T2 (107)	0.71 (0.5-0.86)	-1342.46 (-2329.44-413.94)
T3/4 (215)	0.58 (0.39-0.76)	-400.3 (-1863.14-980.47)
p value	0.0010	0.0013