

Tissue and imaging biomarkers for hypoxia predict poor outcome in endometrial cancer

Supplementary Materials

Supplementary Table S1: Evaluation of HIF-1 α expression using tissue micro arrays shows a significant overlap with evaluation of full sections

TMA	Full sections				<i>P</i>
	Epithelial HIF-1 α (%)		Stromal HIF-1 α (%)		
	Low (<i>n</i>)	High (<i>n</i>)	Low (<i>n</i>)	High (<i>n</i>)	
Epithelial HIF-1 α : Low	88 (14)	12 (2)			0.04
High	46 (6)*	54 (7)			
Stromal HIF-1 α Low			77 (10)	23 (3)	0.003
High			19 (3)	81 (13)	

*Two cases showed heterogeneous expression pattern. Full sections from 29 patients stained for HIF-1 α .

Supplementary Table S2: Gene Ontology (GO) annotated gene sets supplied by the Broad Institute (MSigDB).

Rank	Gene set	<i>P</i>	FDR (%)
1	M_PHASE	0.0	0.0
2	DEFENSE_RESPONSE	0.0	0.0
3	M_PHASE_OF_MITOTIC_CELL_CYCLE	0.0	0.0
4	MITOSIS	0.0	0.0
5	CELL_CYCLE_PHASE	0.0	0.0
6	CELL_CYCLE_PROCESS	0.0	0.0
7	INFLAMMATORY_RESPONSE	0.0	0.0
8	RESPONSE_TO_OTHER_ORGANISM	0.0	0.0
9	IMMUNE_SYSTEM_PROCESS	0.0	0.0
10	MITOTIC_CELL_CYCLE	0.0	0.0

Top ranked gene sets enriched in endometrial (malignant and premalignant) lesions with high expression of stromal HIF-1 α compared to low stromal protein expression.

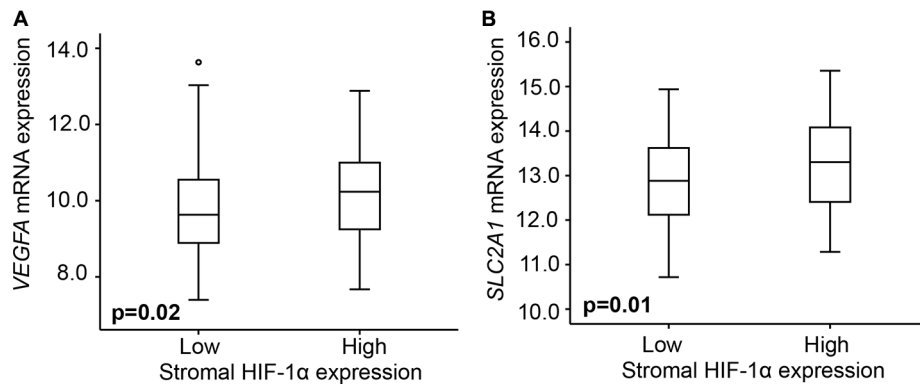
Supplementary Table S3: Top ranked therapeutic agents anti-correlated to lesions expressing high hypoxia signature score versus low hypoxia signature score using Connectivity Map (version 2)

Rank	Compound	<i>N</i>	Enrichment	<i>P</i>	Description
1	LY-294002	61	-0,356	0	PI3K inhibitor
2	Trichostatin A	182	-0,22	0	HDAC inhibitor
3	Tanespimycin (17-AAG)	62	-0,27	0,0002	HSP90 inhibitor

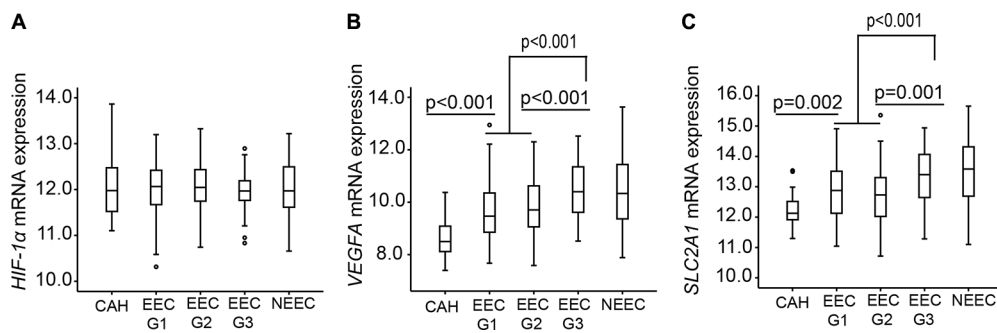
Supplementary Table S4: Methods applied for immunohistochemical staining

Primary antibody	Secondary antibody	Vendor	Buffer ^d	Incubation time	Incubation temperature	Dilution	
ER α	MM ^a	Anti-mouse HRP (K4001)	Dako (M7047)	pH 9	30 min	Room temp.	1:50
PR	MM ^a	Anti-mouse HRP (K4001)	Dako (M3569)	pH 9	60 min	Room temp.	1:150
HIF-1 α	MR ^b	Anti-rabbit HRP (K4002) ^c	Abcam (ab51608)	pH 9	60 min	Room temp.	1:200
CD45	MM ^a	Anti-mouse HRP (K4001)	Dako (M0701)	pH 9	60 min	Room temp.	1:200

^aMonoclonal mouse, ^bMonoclonal rabbit, ^cWhen stained together with CD45 anti-Rabbit AP was used (Southern Biotech, 4050-04), ^dTarget Retrieval Solution, pH9 (Dako, S2367).



Supplementary Figure S1: Gene expression according to stromal HIF-1 α protein expression. Gene expression value of *VEGFA* (A) and *SLC2A1* (B) in primary endometrial cancer (EC) lesions and complex atypical hyperplasia (CAH) according to high and low stromal HIF-1 α protein expression.



Supplementary Figure S2: Gene expression according to histological type and grade. Gene expressions value of *HIF-1 α* (A), *VEGFA* (B) and *SLC2A1* (C) in complex atypical hyperplasia (CAH) patients, endometrioid endometrial cancer (EEC) grade 1, 2 and 3, and non-endometrioid endometrial cancer (NEEC).