

Clinical implications of NRF3 mRNA and Protein expression in ovarian cancer

Mahdi Hosseinzadeh^{1,2}, Zahra Madjd^{3,4,5}, Mohsen Rashid¹, Elham Kalantari³, Behrooz Shokouhi⁶, Manizheh Sayyah-Melli^{7,8}, Mohammad Reza Sadeghi^{1,9*}

¹Department of Molecular Medicine, Faculty of Advanced Medical Sciences, Tabriz University of Medical Sciences, Tabriz, Iran

²Student Research Committee, Tabriz University of Medical Sciences, Tabriz, Iran

³Oncopathology Research Center, Iran University of Medical Sciences (IUMS), Tehran, Iran

⁴Department of Pathology, School of Medicine, Iran University of Medical Sciences, Tehran, Iran

⁵Department of Molecular Medicine, Faculty of Advanced Technologies in Medicine, Iran University of Medical Sciences, Tehran, Iran

⁶Department of Pathology, Tabriz University of Medical Sciences, Tabriz, Iran

⁷Women’s Reproductive Health Research Center, Tabriz University of Medical Sciences, Tabriz, Iran

⁸Fellowship of Gynecologic Oncology. Women's Reproductive Health Research Center, Tabriz University of Medical Sciences, Tabriz, Iran

⁹Research Center for Pharmaceutical Nanotechnology, Biomedicine institute, Tabriz University of Medical Sciences, Tabriz, Iran

*Corresponding author: Mohammad Reza Sadeghi, Email: sadeghimr@tbzmed.ac.ir

Table S1. Specimen Sources

Phase	Center	Location	Type	Details	number
First Phase	Al-Zahra Hospital operating room	Tabriz, Iran	Fresh Frozen samples	Acquisition Period: 2021–2024	29 pairs of tumoral and matched adjacent non-tumoral margins
Second Phase	Kowsar Hospital	Urmia, Iran	FFPE samples	Primary diagnosis of ovarian cancer (OC)	Total: 171 FFPE samples
	Al-Zahra Hospital	Tabriz, Iran	FFPE samples	Primary diagnosis of ovarian cancer (OC)	
	Iran University of Medical Sciences	Tehran, Iran	FFPE samples	Biobank	

Table S2. Primer sequences used for NRF3 and GAPDH in qPCR assays. designed and evaluated using the Oligo7 and Primer3 software.

Gene	Primer Type	Sequence (5' → 3')	Notes
NRF3	Forward	ATG TGC GAG CGA GGA GAA TG	Exon–exon junction
NRF3	Reverse	TTC TGG GCT GAC ACC CTT TC	Normal primer
GAPDH	Forward	GAA GGT GAA GGT CGG AGT C	Common housekeeping gene primer
GAPDH	Reverse	GAA GAT GGT GAT GGG ATT TC	Widely used reference primer

Table S3. Functional enrichment of NRF3 (NFE2L3) network (STRING analysis).

Rank	Pathway / GO Term	Category	Genes in Network	Total Genes in Pathway	Fold Enrichment	<i>FDR</i> ¹ -adjusted p-value
1	Integrated stress response signaling (GO:0140467)	GO Biological Process	4	31	2.5	9.10e-06
2	RNA polymerase II transcription regulator complex (GO:0090575)	GO Cellular Component	7	256	1.83	1.13e-09
3	Transcription factor AP-1 complex (GO:0035976)	GO Cellular Component	3	5	3.17	1.68e-06
4	IL-17 signaling pathway (hsa04657)	KEGG Pathway	3	91	1.91	0.0016
5	Osteoclast differentiation (hsa04380)	KEGG Pathway	3	120	1.79	0.0016

Table S4. Top transcription factors enriched for NRF3 interactors using Enrichr across ChEA, ARCHS4, and TRRUST.

Database	Top TFs (examples)	Adjusted p-value range	Biological relevance
ChEA 2022	EGR1, BRD4, EP300, HIF1A, GATA1/2, MAF	0.0004–0.0097	Stress response, chromatin regulation
ARCHS4 Co-expression	ZFP36L1, NR4A1, JUND, KLF4, ATF3, JUNB	0.0066–0.058	Co-expression in stress/cancer programs
TRRUST 2019	HDAC3, FOSL1, JUN, ESR2, ARNT, RB1, HDAC4	0.0001–0.0023	AP-1 complex, estrogen signaling, epigenetic control

Table S5. stratified Cox regression for PFS and OS where FIGO stage was used as the stratification variable and NRF3 expression was tested as a covariate

Endpoint	Covariate	PFS-HR (95% CI)	95% CI for HR	p-value	sig
OS	NRF3 expression	2.089	0.723 – 6.041	0.174	ns
PFS	NRF3 expression	1.848	0.914 – 3.738	0.087	BDL

¹ False Discovery Rate

Fig. S1. Histological composition of study samples.

