TANDEM HISTONE METHYLTRANSFERASE UPREGULATION DEFINES A UNIQUE AGGRESSIVE PROSTATE CANCER PHENOTYPE

Mikolaj Filon*, Joseph Gawdzik, Andrew Truong, Bing Yang, Rehaan Machhi, and David F Jarrard

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Department of Urology
UNIVERSITY OF WISCONSIN
SCHOOL OF MEDICINE AND PUBLIC HEALTH

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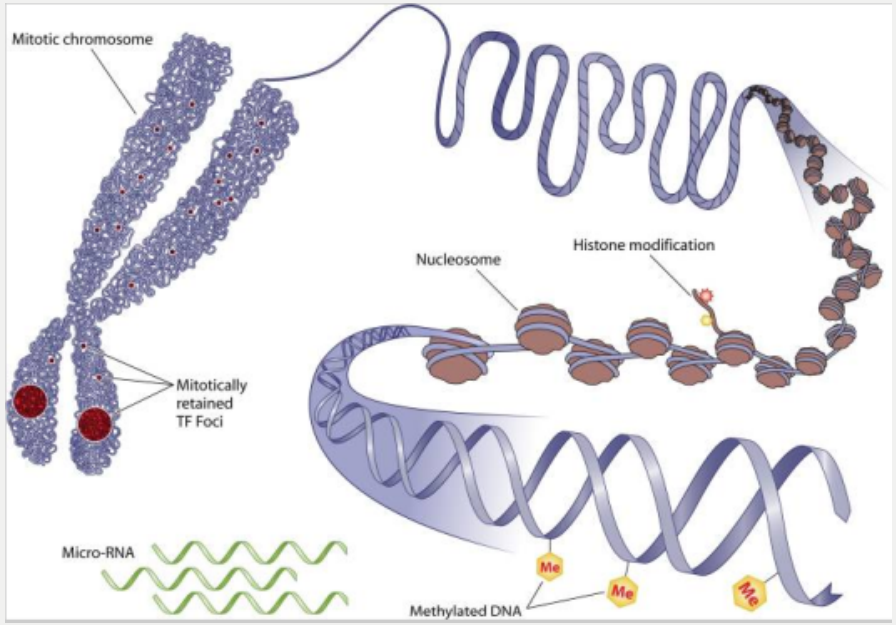
Background

- Epigenetic dysregulation ubiquitous in cancer
- Histone Methyltransferases (HMT) have been implicated in the progression of prostate cancer
- Enhancer of Zeste Homolog 2 (EZH2)
  - H3K27 tri-methylation, transcriptional silencer
  - Upregulated in cancer, acting on tumor suppressors
- Nuclear SET Domain 2 (NSD2)
  - H3K36 di-methylation, active chromatin
  - Down-stream “effector” of EZH2
- Co-regulation
  - miRNA repression of NSD2 lifted by EZH2



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Aims

1. Determine trends in EZH2 and NSD2 protein levels with disease progression and change in hormone status using patient-derived tissue samples

2. Investigate association between EZH2 and NSD2 in cancer genomic libraries, and compare their predictive ability of biochemical recurrence

Methods

1. Determine trends in EZH2 and NSD2 protein levels with disease progression and change in hormone status using patient-derived tissue samples
   a. VECTRA immunohistochemistry staining with cell segmentation
      i. Hormone Responsiveness TMA (hrTMA): (n=28)
         a) Hormone sensitive vs hormone resistant cancer tissue
      ii. Progression TMA (pTMA): cores from radical prostatectomy (n=71)
         a) Benign, primary, and metastatic tissue comparison
2. Investigate association between EZH2 and NSD2 in cancer genomic libraries, and compare their predictive ability of biochemical recurrence
   a. Accessed cBioPortal, utilizing TCGA (n=498) and MSKCC (n=140) datasets
      i. Correlation analysis, univariate and multivariate statistics
      ii. Kaplan Meier comparison of Disease free survival

Results

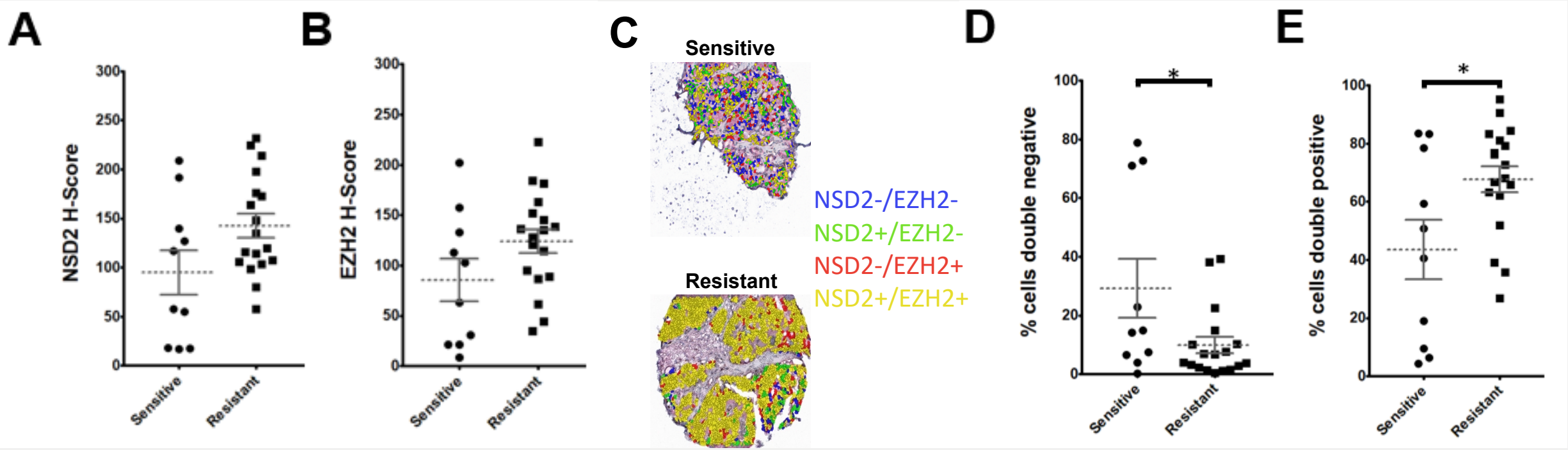

Figure 1. Concurrent immunostaining demonstrates percentage of cells with colocalization of NSD2 and EZH2 significantly increases in hormone resistant prostate cancer. Immunochemistry was quantitated using VECTRA and inform software for hormone responsiveness patient tissue microarray. (A-B) Mean H-score of NSD2 and EZH2 (C) Color-graphic representation of VECTRA immunostaining combinations for NSD2 and EZH2 in individual epithelial cells (D) Percentage of individual epithelial cells negative for both NSD2 and EZH2 signals (E) Percentage of individual epithelial cells positive for both NSD2 and EZH2 signals compared between hormone and resistant PCa. ($p<0.05$ indicated by *, <0.01 indicated by ** and <0.001 indicated by ***).

Results

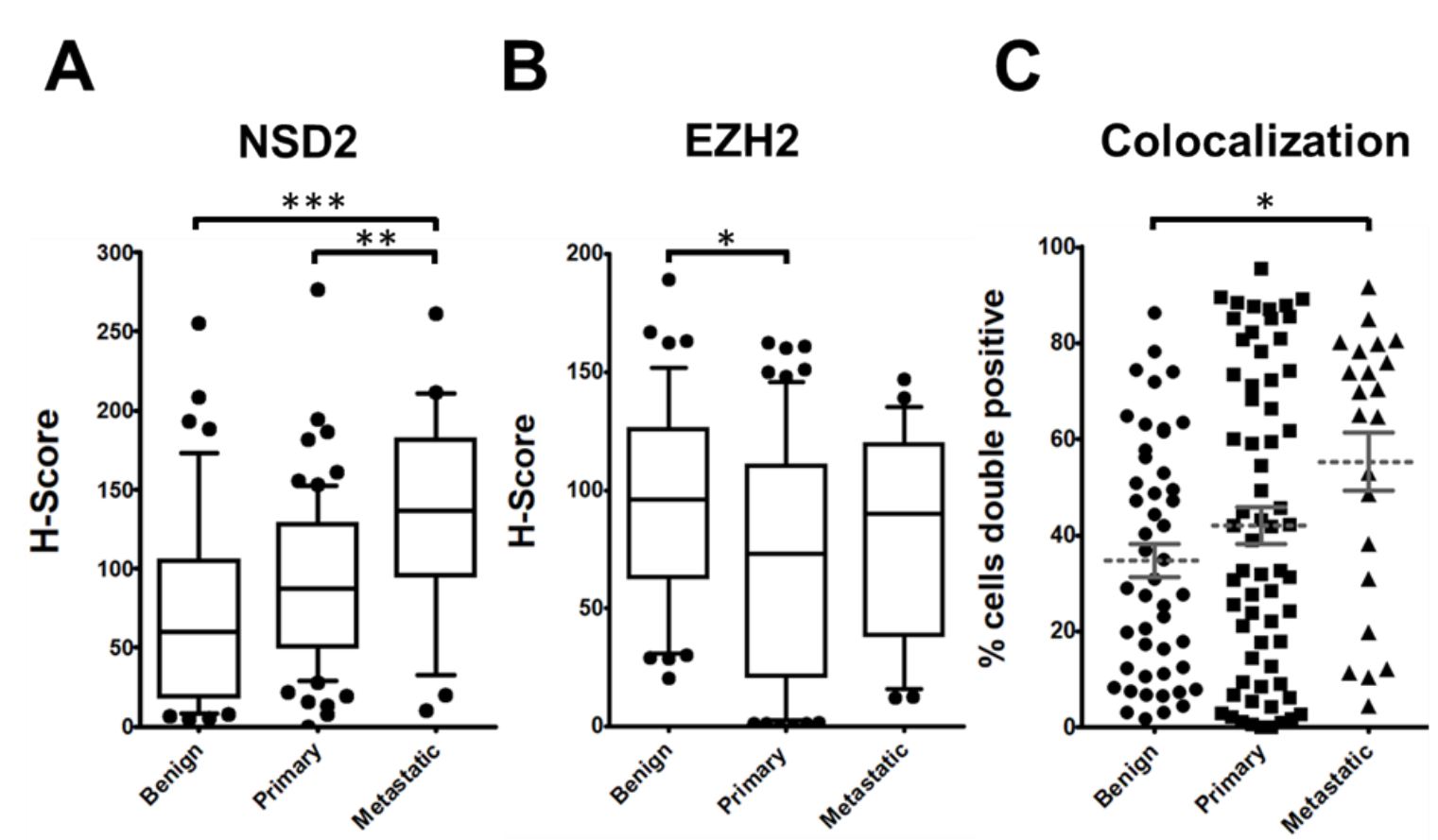

Figure 2. Concurrent immunostaining demonstrates NSD2 increases and EZH2 decreases during prostate cancer progression. (A-B) H-scores of NSD2 and EZH2 compared between benign, primary, and metastatic PCa radical prostatectomy cores. (C) Percentage of individual cells staining positive for both EZH2 and NSD2 signals in benign, primary, and metastatic PCa ($p<0.05$ indicated by *, <0.01 indicated by ** and <0.001 indicated by ***).

Results

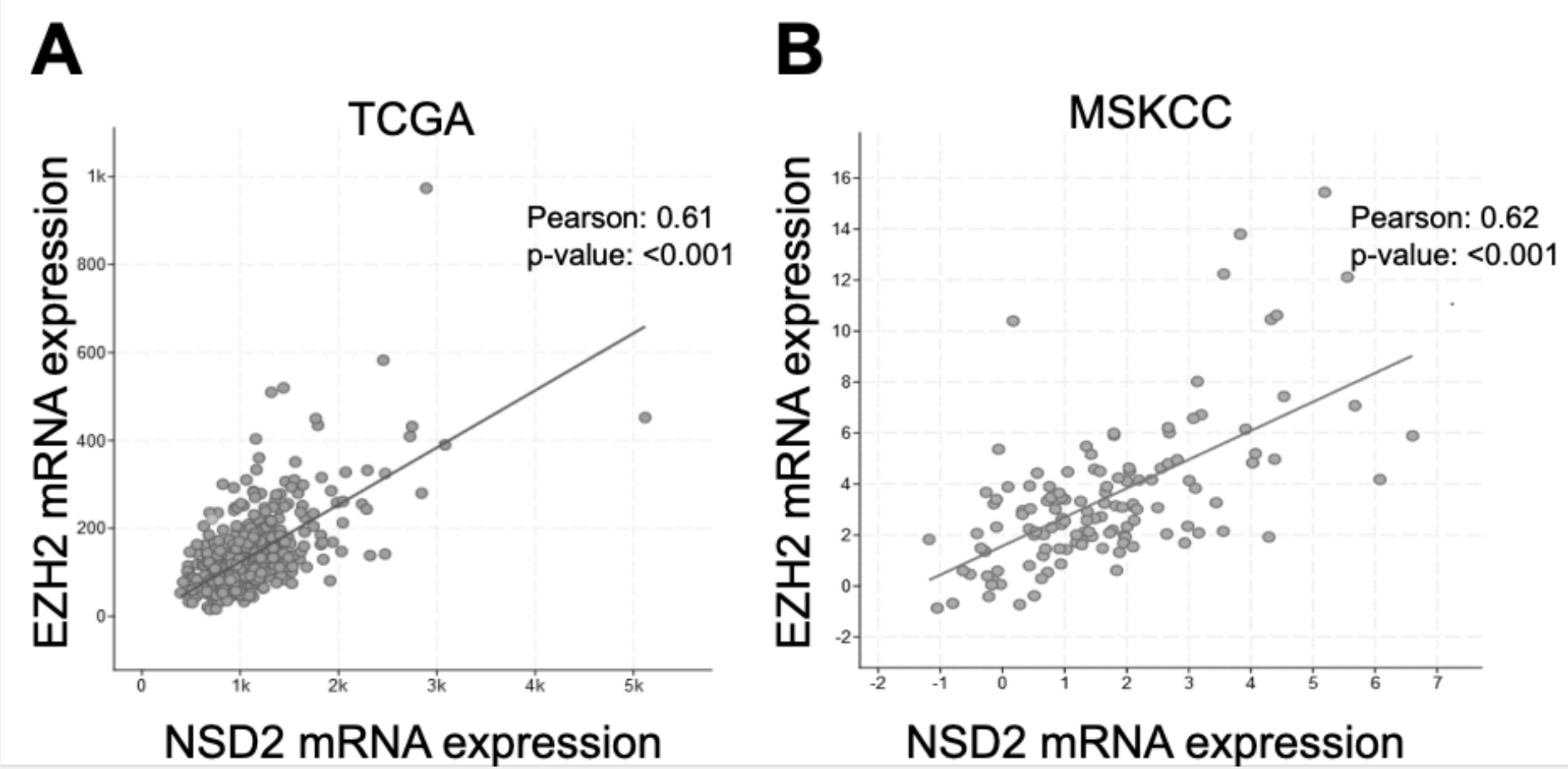

Figure 3. Prostate cancer database expression data demonstrates significant EZH2 and NSD2 coexpression. For individual tissue samples with expression for both enzymes available, mRNA expression values plotted for each marker. (A) TCGA (n=491) (B) MSKCC (n=128). mRNA expression data were queried and downloaded using cBioPortal platform. Pearson coefficient documented.

Results

Table 1. Association of NSD2, EZH2 RNA expression with patient pathologic features

	MSKCC					TCGA				
Variable	**n**	**NSD2**	**p-value**	**EZH2**	**p-value**	**n**	**NSD2**	**p-value**	**EZH2**	**p-value**
Grade Group										
1-2	94	6.76 (0.28)		9.72 (0.11)		191	937 (233)		100 (42)	
3-5	44	6.95 (0.46)	0.003	9.77 (0.14)	0.017	307	1185 (480)	<0.001	157 (101)	<0.001
Stage										
2	86	6.78 (0.27)		9.72 (0.10)		187	969 (255)		110 (53)	
3	47	6.88 (0.46)		9.75 (0.14)		293	1137 (401)		147 (84)	
4	7	6.94 (0.48)	0.22	9.79 (0.11)	0.09	11	1796 (1295)	<0.001	225 (179)	<0.001
SV involved										
No	116	6.80 (0.30)		9.73 (0.11)		345	1018 (331)		121 (68)	
Yes	17	6.90 (0.59)	0.31	9.75 (0.17)	0.50	135	1208 (395)	<0.001	162 (86)	<0.001

Data reported as mean (SD)

Results

Table 2. Univariate cox regression analysis for predicting biochemical recurrence

Variable	MSKCC Haz. Ratio	MSKCC 95% Conf. interval	MSKCC p-value	TCGA Haz. Ratio	TCGA 95% Conf. interval	TCGA p-value
Grade Group Category*	10.6	4.9-22.9	<0.001	6.2	2.7-14.5	<0.001
Stage	3.4	2.1-5.4	<0.001	3.3	2.0-5.6	<0.001
SV involvement	7.0	3.4-14.5	<0.001	3.4	2.0-5.7	<0.001
Top Quartile NSD2	3.5	1.8-6.8	<0.001	2.7	1.6-4.5	<0.001
Top Quartile EZH2	2.4	1.2-4.7	0.016	2.9	1.7-4.9	<0.001
Top Quartile NSD2 and EZH2	2.9	1.4-6.1	0.008	2.8	1.6-4.9	0.001

*Grade Group Categories: (1-2) vs (3-5)

Table 3. Multivariate cox regression analysis

Variables	Haz. Ratio	95% Conf. interval	p-value
TCGA			
Top Quartile NSD2	1.9	1.1-3.1	0.022
*Grade Group Category	3.7	1.5-9.0	0.004
Stage	2.2	1.2-4.0	0.007
Top Quartile EZH2	1.9	1.1-3.2	0.017
Grade Group Category	3.4	1.4-8.3	0.008
Stage	2.4	1.4-4.3	0.003
Top Quartile NSD2 and EZH2	1.9	1.1-3.5	0.023
Grade Group Category	5.3	2.3-15.5	<0.001
MSKCC			
Top Quartile EZH2	2.4	1.2-4.9	0.012
Grade Group Category	9.2	4.1-20.7	<0.001
Stage	2.9	1.7-5.1	<0.001
Top Quartile NSD2 and EZH2	2.6	1.2-5.5	0.014
Grade Group Category	8.3	3.7-18.7	<0.001
Stage	2.8	1.6-4.8	<0.001

Remained in model (p<0.05)
**Grade Group Categories: (1-2) vs (3-5)*

Results

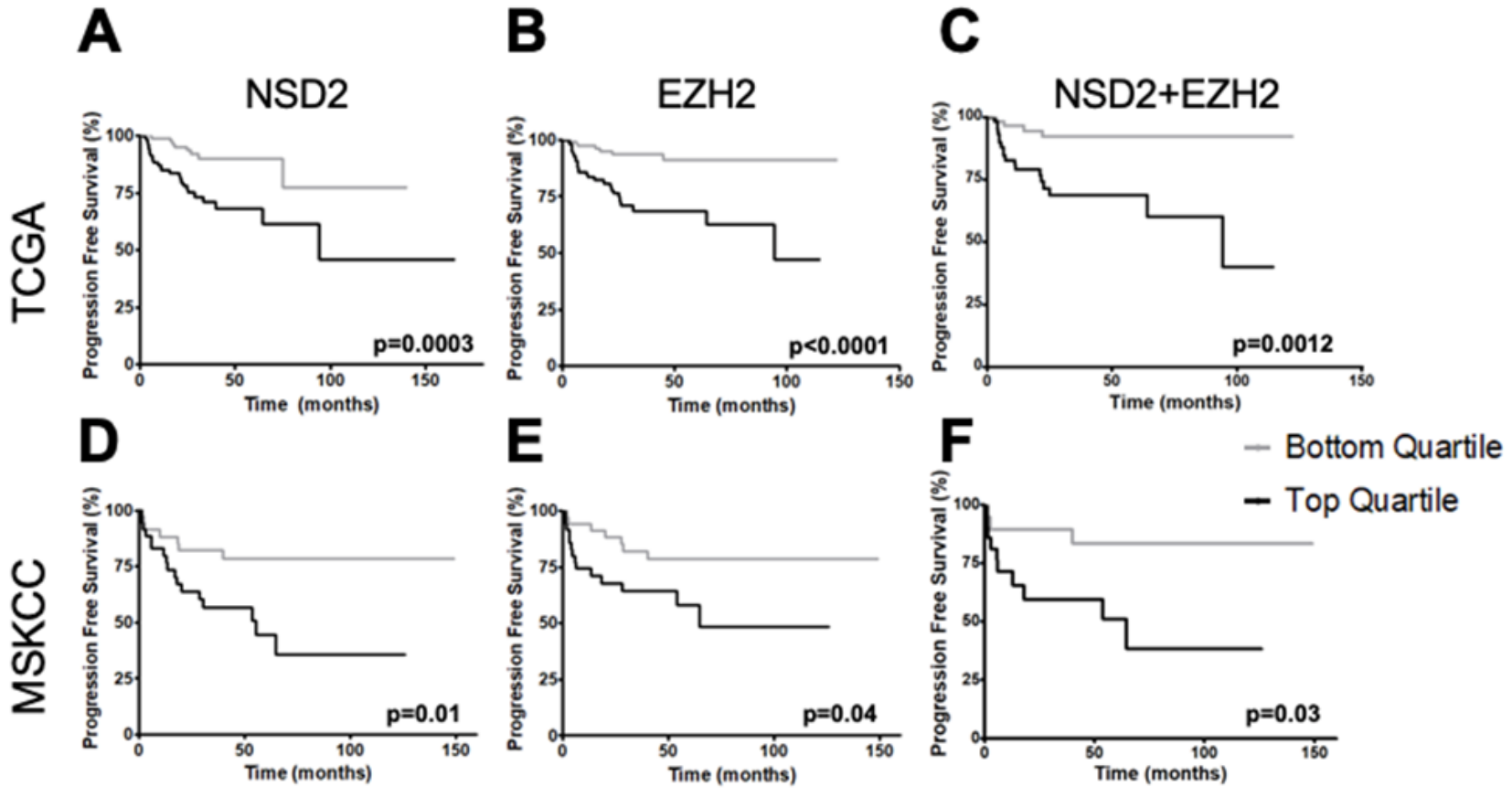

Figure 4. Top Quartile NSD2 or EZH2 expression predicts biochemical recurrence in Gleason Grade 3-5 disease. Kaplan Meier analysis performed using clinical outcome data available on cBioPortal for (A-C) TCGA and (D-F) MSKCC. Biochemical recurrence reported as disease free time determined by PSA. In each dataset, samples were assigned individual quartile rank for NSD2, EZH2, as well as their sum. Survival analysis comparing the Gleason Grade 3-5 samples in the top quartile to those in the bottom quartile was performed for (A,D) NSD2 (B,E) EZH2 and (C,F) their sum. p-values are documented.

Conclusions

- Neither NSD2 nor EZH2 expression increased with hormone status (p=0.051, 0.09)
  - Percentage of cells expressing both NSD2/EZH2 significant (p=0.02)
- NSD2 protein levels and Colocalization of NSD2/EZH2 significantly increased in metastatic disease
- Tight correlation between EZH2 and NSD2 RNA expression
  - Association with GG, stage, and SV involvement
  - Top quartile in expression predicts biochemical recurrence
    - EZH2, NSD2 alone similar to top quartile in both
- Subset of patients may be amenable to therapeutic targeting