Correlations between Gene Amplification and Protein Expression of Topoisomerase 2A (TOP2A) in Squamous Cell Carcinoma of the Lung

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ABSTRACT

Background: While DNA topoisomerase 2A (TOP2A) plays an essential role in maintaining the structural integrity of the double helix during replication and recombination, excessive expression of this enzyme may promote malignant cell transformations. In fact, increased levels of TOP2A have been observed in various cancer cell lines including squamous cell carcinoma of the lung. This study sought to identify correlations between genotypic and phenotypic evidence of TOP2A obtained via in situ hybridization (ISH) and immunohistochemistry (IHC) techniques.

Methods Tissue microarrays created from 29 samples of Stage I Squamous Cell Carcinoma of the lung were stained with VENTANA BenchMark ULTRA platform with dual color ISH molecular probes TOP2A / CEP17 and antiTOP2A antibody (clone JS5B4-rabbit monoclonal antibody). Gene copy numbers were analyzed using bright field microscopy. Gene amplification was considered in cells exhibiting gene copies > 3 or TOP2A:CEP17 ratios > 1.82. IHC stains were quantified using Spectrum software (Apeiro technologies) using the nuclear algorithm. All levels of protein expression (+1 to +3) were considered positive.
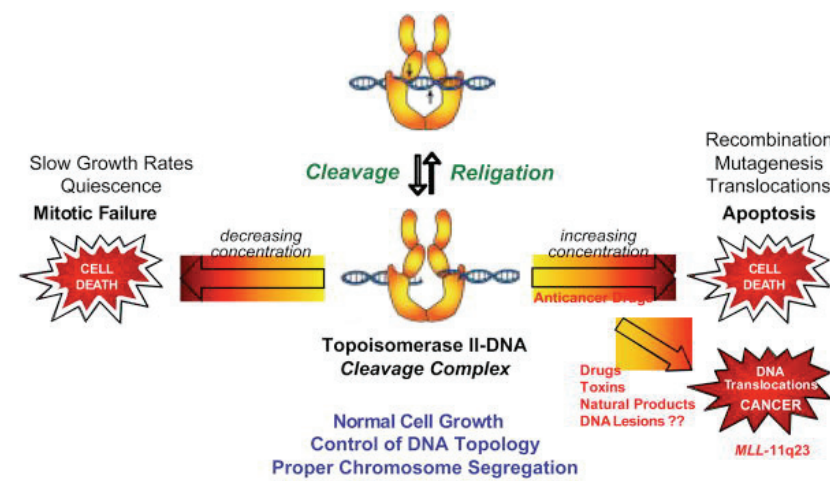
Results: A moderate Pearson Correlation (0.4) between TOP2A gene amplification and protein expression was identified.

Conclusion: While gene amplification moderately correlated with protein expression of TOP2A, additional factors influencing protein expression independently of gene amplification should be identified.

INTRODUCTION

While DNA topoisomerase 2A (TOP2A) plays an essential role in maintaining the structural integrity of the double helix during replication and recombination, excessive expression of this enzyme may promote malignant cell transformations.

TOP2A protein expression has been correlated with poorer prognoses in breast cancer, as well as small cell and squamous cell carcinomas of the lung. TOP2A gene amplification has been correlated with increased responses and reduced resistance to targeted therapies for breast cancer. However, the relationship between gene amplification and protein expression in squamous cell carcinoma is unknown.
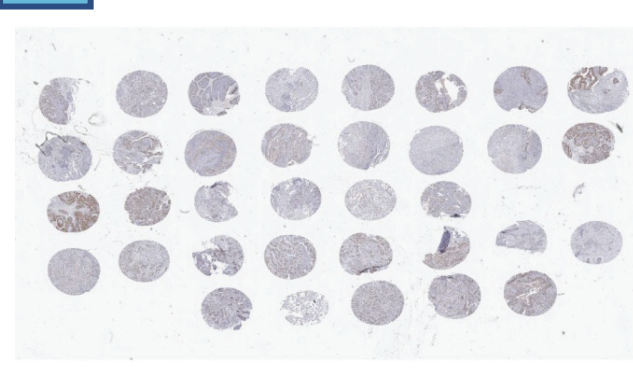

McClendon, A.K., Osheroff, N., 2007

CONCLUSIONS

- TOP2A protein expression moderately correlates with TOP2A gene amplification in squamous cell carcinoma of the lung (Pearson Correlation: 0.4).
- Additional factors influencing the TOP2A protein expression independently of TOP2A gene amplification should be identified.
- Verification of TOP2A protein expression may not substitute for that of TOP2A gene amplification.

METHODS

1 Tissue microarrays (TMAs) were created using 29 tissue samples of Stage I Squamous Cell Carcinoma of the Lung. Cores were resected at TJUH in 1996/97.

2 *In situ hybridization (ISH) identified nuclear TOP2A gene copies, centromeres.*

TOP2A (gene copies)
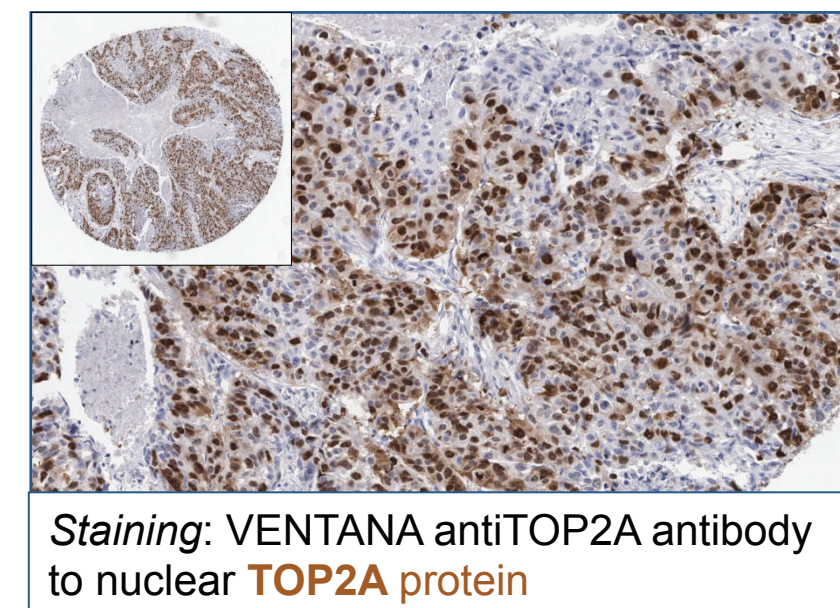**CEP17** (centromeres)

Staining:
- VENTANA BenchMark ULTRA platform
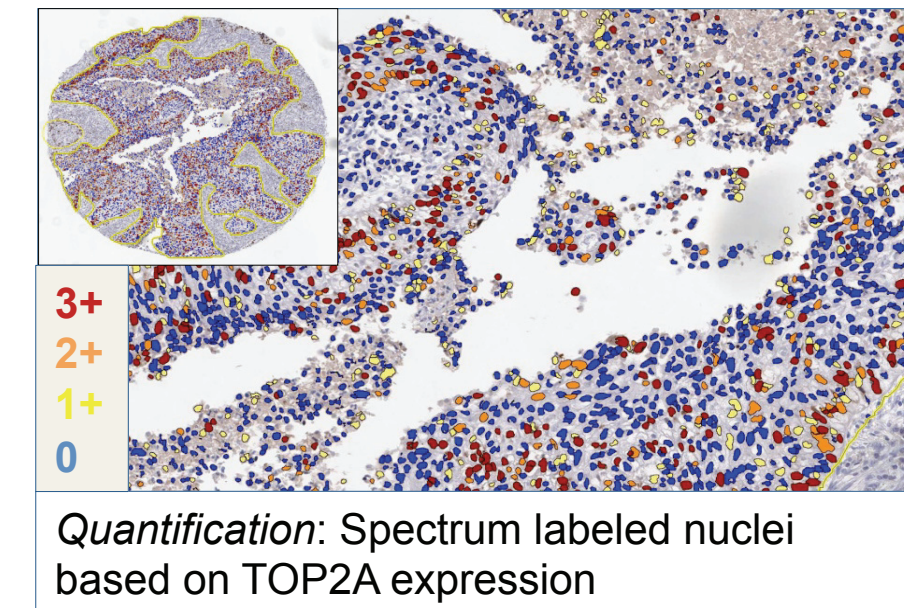- Dual color ISH molecular probes

Quantification:
- Bright field microscopy: 20-40 cells quantified per section
- Recorded values per cell for each section: Average number of gene copies
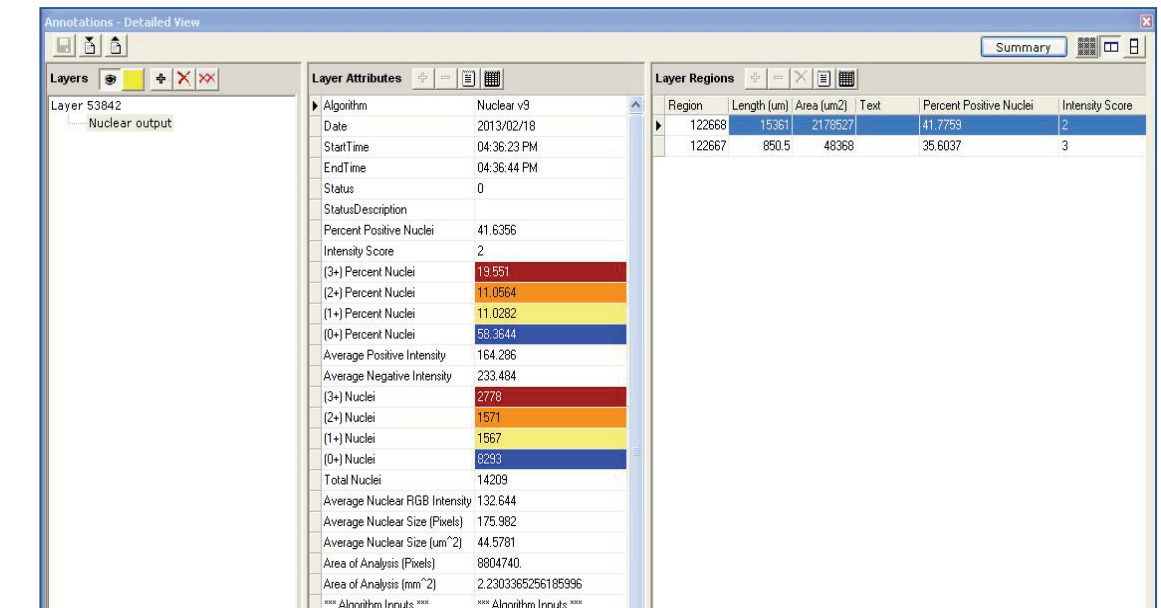
TOP2A:CEP17 ratio

3 *Immunohistochemistry (IHC) detected nuclear TOP2A protein expression, quantified with Spectrum Software (Apeiro technologies).*

Staining: VENTANA antiTOP2A antibody to nuclear **TOP2A** protein

3+
2+
1+
0

Quantification: Spectrum labeled nuclei based on TOP2A expression

RESULTS

Statistical analyses were conducted on the data obtained through the staining and quantification processes and compiled as shown in the following tables. A moderate Pearson Correlation (0.4) was observed.

TMA2		3+ (%)	2+ (%)	1+ (%)	0 (%)	3+, 2+, 1+	3+, 2+	Gene Copy	Ratio
4AVG 10	21	2.70	3.12	4.25	89.93	10.07	5.81	1.8	0.73
AVG 5	5	2.07	2.42	3.21	92.31	7.69	4.49	2.18	0.86
6AVG 6	27	17.92	7.46	6.35	68.26	31.74	25.39	2	1
4AVG 1	12	3.27	2.67	3.69	90.37	9.63	5.94	2.2	1.04
4AVG 13	24	11.98	8.05	8.03	71.93	28.07	20.04	3.1	1.05
AVG 4	4	10.62	13.76	13.91	61.72	38.28	24.37	2.13	1.06
4AVG 11	10	4.10	1.59	2.06	92.25	7.75	5.68	2.28	1.06
4AVG 7	18	13.67	5.26	4.80	76.26	23.74	18.93	2.1	1.08
4AVG 12	23	8.26	5.01	4.48	82.26	17.74	13.27	2.4	1.09
6AVG 5	26	7.31	4.23	6.87	81.59	18.41	11.54	3	1.09
4AVG 11	22	18.02	7.51	6.88	67.59	32.41	25.53	2.15	1.1
6AVG 7	28	18.56	10.01	10.48	60.96	39.04	28.57	2.55	1.13
6AVG 4	15	22.10	5.12	5.06	67.71	32.29	27.22	2.8	1.23
AVG 9	30	22.95	6.57	4.73	65.75	34.25	29.52	3.9	1.3
4AVG 9	20	10.30	7.33	5.41	76.96	23.04	17.63	3.2	1.35
4AVG 13	11	14.86	9.22	9.45	66.48	33.52	24.08	3.93	1.44
6AVG 8	29	14.07	5.51	6.01	74.42	25.58	19.57	3.45	1.44
AVG 2	2	9.41	6.30	6.23	78.06	21.94	15.71	2.68	1.49
4AVG 8	19	15.03	6.46	5.81	72.71	27.29	21.49	2.88	1.49
4AVG 5	16	10.37	6.26	4.98	78.39	21.61	16.63	3.45	1.55
4AVG 10	9	21.38	10.43	6.97	61.26	38.78	31.81	4.23	1.58
4AVG 1	1	12.60	7.06	6.35	73.66	26.01	19.66	3.85	1.77
6AVG 6	6	25.72	16.57	11.43	46.28	53.72	42.29	4.15	1.82
4AVG 6	17	1.03	0.72	2.53	95.73	4.27	1.75	4.15	1.82
4AVG 3	14	21.90	16.08	14.52	47.50	52.50	37.98	4.15	2.27
4AVG 2	13	13.51	8.77	8.66	69.06	30.94	22.28	5.45	2.63
AVG 7	7	10.66	3.34	3.16	82.84	17.16	14.00	7.95	2.72
AVG 8	8	12.82	9.61	8.80	68.77	31.23	22.43	8.48	3.73
AVG 3	3	29.25	11.88	10.19	48.68	51.32	41.13	8.48	4.24

Gene amplification: Gene copies > 3, **TOP2A:CEP17** > 1.82

Protein expression: **TOP2A** protein > 0

In both tables to the right, TOP2A-amplified tumor cells were positive for nuclear TOP2A protein at all levels of expression more consistently than non-amplified tumor cells. This trend is especially pronounced when comparing high levels of protein expression between amplified and non-amplified cells on the basis of TOP2A:CEP17 ratios (Table 2). However, some cases that didn't demonstrate TOP2A gene amplification expressed TOP2A protein and vice versa.

Protein Expression	Gene Copies > 3 (n = 16)	Gene Copies < 3 (n = 13)
> 10%	94%	77%
> 20%	81%	62%
> 30%	50%	38%

Table 1

Protein Expression	Ratio > 1.82 (n = 7)	Ratio < 1.82 (n = 22)
> 10%	86%	86%
> 20%	71%	45%
> 30%	71%	36%

Table 2