Expression of Class III beta-tubulin (TUBB3) in 3580 colorectal cancers (CRCs) and correlation with clinico-pathological and molecular features

[1]Joanne Xiu, [1]Sandeep Reddy, [2]Wafik S. El-Deiry
[1]Caris Life Sciences, Phoenix, AZ [2]Fox Chase Cancer Center, Philadelphia, PA

Abstract#4923

Background: Class III beta-tubulin plays a crucial role in intracellular transport, meiosis and mitosis. High expression of TUBB3 has been shown to associate with poor prognosis and taxane resistance in various cancer types. CRC is known to be generally resistant to taxane therapy. We investigated expression of TUBB3 in 3580 CRCs and made correlations with clinico-pathological and molecular parameters.

Methods: 3580 CRC samples were evaluated by tumor profiling (Caris Life Sciences, Phoenix, AZ). Tests included Sanger or next generation sequencing (NGS), protein expression by immunohistochemistry (IHC) and gene amplification by in situ hybridization (ISH). TUBB3 expression was evaluated by immunohistochemistry (Ab: POLY, Covance) and expression higher than 2+, 30% was scored as positive.

Results: TUBB3 positive expression was observed in 37% (1320/3580) of the complete CRC cohort, specifically 27% in mucinous histology (164/617) and 17% in signet ring histology (29/171). While the expression was not associated with average patient age (59 years old) or gender (53% vs. 50% male), TUBB3 was more frequently expressed in tumors that originated from the left colon (370/1016 or 36%) than from the right colon (235/790 or 30%, p=0.003). In the 1847 tumors taken from metastatic sites, 40% (746) overexpressed TUBB3 while in 1629 CRCs taken from primary tumors, only 34% (547) overexpressed TUBB3 (p<0.0001). Interestingly, in tumors that overexpressed TUBB3, 65% also overexpressed cMET (828/1275) and 33% also overexpressed TLE3 (431/1299). This compares to 50% cMET expression (1096/2207, p<0.0001) and 23% TLE3 expression (517/2225, p<0.0001) in tumors that were negative for TUBB3. Microsatellite instability detected by fragment analysis was more prevalent in the TUBB3negative cohort than the TUBB3positive cohort (7.4% or 77/1046 vs. 2.9% or 16/558, p=0.0002). Mutations in APC (62% vs. 56%, p=0.0003) and KRAS (55% vs. 46%, p<0.0001) were more frequent in TUBB3positive tumors, while GNAS (2% vs. 5%, p<0.0001) and SMAD4 (10.1% vs. 14.6%, p=0.0005) mutations were significantly more frequent in tumors that were negative for TUBB3 expression.

Conclusions: High expression of TUBB3 was found in 37% of CRCs, and was significantly associated with tumors that originated from the left colon and with tumors taken from metastatic sites. Distinct biomarker features detected by IHC and sequencing suggest that TUBB3 expression may carry theranostic importance which warrants further investigation in clinical trials.

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Background:

Class III beta-tubulin is a microtubule protein, normally expressed in cells of neuronal origin as well as extra-neuronal cells and is involved in crucial cellular functions; it's also found to be overexpressed in several solid tumors, including non-small-cell lung, ovarian, urothelial, prostate, head and neck squamous cancers, etc.

Taxanes prevent microtubule de-polymerization and chromosome segregation at mitosis, and high TUBB3 expression has been shown to be a resistance mechanism in NSCLC, ovarian and prostate cancer. The activity of taxanes have been disappointing in CRC: in the case of docetaxel, in the two published phase II studies, only 1 partial response was observed in the 35 patients treated, and for paclitaxel, the phase II trial shows no response in 15 assessable patients. However, in these trials, no molecular stratification was implemented for patient selection. Potential resistance mechanisms proposed for taxane resistance in colorectal cancer included chromosomal instability and spindle checkpoint abnormality, however these have not been validated in clinical trials.

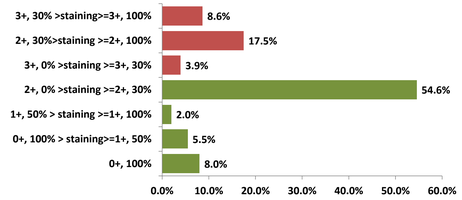
The expression of TUBB3 is systemically evaluated in a large cohort of CRC tumors in our study. The information is important for clinical trials to select CRC patients who will respond to taxane therapies.

Results:

1. Patient characteristics

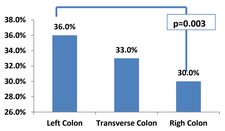
		Count N
Tumor Origin	Left Colon	1016
	Righ Colon	790
	Transverse Colon	61
	Not specified	1713
Gender	Male	1843
	female	1737
Age	Average Age	58.7
Histology	Mucinous	617
	Signet Ring	171
	squamous	12
	adenocarcinoma, not specified	2852
Total		3580

2. TUBB3 staining details in the complete cohort of 3580 colorectal tumors. The staining results on the Y axis include the staining intensity (1+, 2+ or 3+) and staining percentage (0%-100%). Red: positive results using the threshold of 2+, 30%; green: negative results.



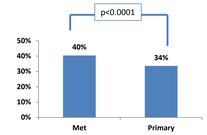
Results:

3. TUBB3 expression is higher in tumors originating from the left colon.



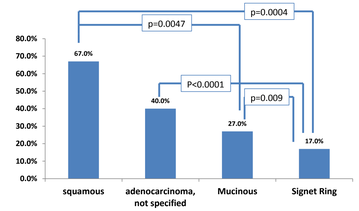
Tumor origin	TUBB3+		Total
Left Colon	370	36%	1016
Transverse Colon	20	33%	61
Righ Colon	235	30%	790
Not specified	695	41%	1713

4. TUBB3 expression is higher in metastases vs. primary tumors



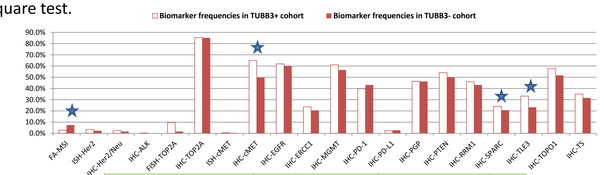
	TUBB3+		Total
Met	746	40%	1847
Primary	547	34%	1629
Not specified	27	26%	104

5. Differential expression of TUBB3 in histological subgroups.



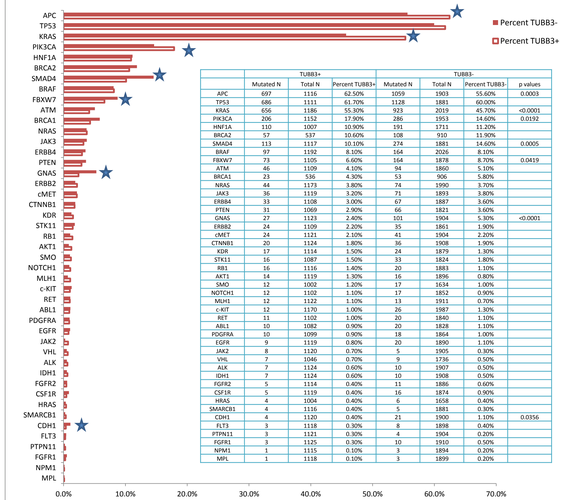
Histology	TUBB3+		Total
squamous	8	67%	12
adenocarcinoma, not specified	1129	40%	2852
Mucinous	164	27%	617
Signet Ring	29	17%	171

6. Molecular profile comparison of TUBB3 positive and negative CRC tumors tested by IHC and ISH. A star indicates the differences are statistically significant (p<0.05) by Chi-square test.



	TUBB3+			TUBB3-			p values
	Positive N	Total N	Percent TUBB3+	Positive N	Total N	Percent TUBB3-	
FA-MSI	16	558	2.9%	77	1046	7.4%	0.0002
ISH-Her2	40	1168	3.4%	46	1982	2.3%	
IHC-Her2/Neu	32	1306	2.5%	36	2239	1.6%	
IHC-ALK	1	361	0.3%	0	606	0.0%	
FISH-TOP2A	4	42	9.5%	1	63	1.6%	
IHC-TOP2A	1084	1269	85.4%	1858	2183	85.1%	
ISH-cMET	5	1002	0.5%	6	1691	0.4%	
IHC-cMET	828	1275	64.9%	1096	2207	49.7%	<0.0001
IHC-EGFR	431	696	61.9%	712	1189	59.9%	
IHC-ERCC1	102	431	23.7%	140	686	20.4%	
IHC-MGMT	795	1303	61.0%	1257	2223	56.5%	
IHC-PD-1	347	871	39.8%	638	1479	43.1%	
IHC-PD-L1	21	873	2.4%	41	1487	2.8%	
IHC-PGP	588	1266	46.4%	1010	2182	46.3%	
IHC-PTEN	696	1287	54.1%	1108	2221	49.9%	
IHC-RRM1	481	1046	46.0%	792	1838	43.1%	
IHC-SPARC	228	949	24.0%	339	1639	20.7%	0.0487
IHC-TLE3	431	1299	33.2%	517	2225	23.2%	<0.0001
IHC-TOPO1	747	1294	57.7%	1131	2188	51.7%	
IHC-TS	460	1314	35.0%	707	2244	31.5%	

7. Molecular profile comparison of TUBB3 positive and negative CRC tumors tested by sequencing. A star indicates the differences are statistically significant (p<0.05) by Chi-square test.



	TUBB3+			TUBB3-			
	Mutated N	Total N	Percent TUBB3+	Mutated N	Total N	Percent TUBB3-	p values
APC	697	1116	62.50%	1059	1903	55.60%	0.0003
TP53	686	1111	61.70%	1128	1881	60.00%	
KRAS	656	1186	55.30%	923	2019	45.70%	<0.0001
PIK3CA	206	1152	17.90%	286	1953	14.60%	0.0192
HNF1A	110	1007	10.90%	191	1711	11.20%	
BRCA2	57	537	10.60%	108	910	11.90%	
SMAD4	113	1117	10.10%	274	1881	14.60%	0.0005
BRAF	97	1192	8.10%	164	2026	8.10%	
FBXW7	73	1105	6.60%	164	1878	8.70%	0.0419
ATM	46	1109	4.10%	94	1860	5.10%	
BRCA1	23	536	4.30%	53	906	5.80%	
NRAS	44	1173	3.80%	74	1990	3.70%	
JAK3	36	1119	3.20%	71	1893	3.80%	
ERBB4	33	1108	3.00%	67	1887	3.60%	
PTEN	31	1069	2.90%	66	1821	3.60%	
GNAS	27	1123	2.40%	101	1904	5.30%	<0.0001
ERBB2	24	1109	2.20%	35	1861	1.90%	
cMET	24	1121	2.10%	41	1904	2.20%	
CTNNB1	20	1124	1.80%	36	1908	1.90%	
KDR	17	1114	1.50%	24	1879	1.30%	
STK11	16	1087	1.50%	33	1824	1.80%	
RB1	16	1116	1.40%	20	1883	1.10%	
AXT1	14	1119	1.30%	16	1896	0.80%	
SMO	12	1002	1.20%	17	1634	1.00%	
NOTCH1	12	1102	1.10%	17	1852	0.90%	
MLH1	12	1122	1.10%	13	1911	0.70%	
c-KIT	12	1170	1.00%	26	1987	1.30%	
RET	11	1102	1.00%	20	1840	1.10%	
ABL1	10	1082	0.90%	20	1828	1.10%	
PDGFRA	10	1099	0.90%	18	1864	1.00%	
EGFR	9	1119	0.80%	20	1890	1.10%	
JAK2	8	1120	0.70%	5	1905	0.30%	
VHL	7	1046	0.70%	9	1736	0.50%	
ALK	7	1124	0.60%	10	1907	0.50%	
IDH1	7	1124	0.60%	10	1908	0.50%	
FGFR2	5	1114	0.40%	11	1886	0.60%	
CSF1R	5	1119	0.40%	16	1874	0.90%	
HRAS	4	1004	0.40%	6	1658	0.40%	
SMARCB1	4	1116	0.40%	5	1881	0.30%	
CDH1	4	1120	0.40%	21	1900	1.10%	0.0356
FLT3	3	1118	0.30%	8	1898	0.40%	
PTPN11	3	1121	0.30%	4	1904	0.20%	
FGFR1	3	1125	0.30%	10	1910	0.50%	
NPM1	1	1115	0.10%	3	1894	0.20%	
MPL	1	1118	0.10%	3	1899	0.20%	

Conclusions

- Immunohistochemical staining of TUBB3 reveals overexpression in 37% of 3580 CRC tumors, with significantly higher prevalence in tumors originating from the left colon and tumors taken from metastatic sites.
- There are histological differences of TUBB3 expression in colorectal cancer, with the highest expression seen in squamous cell tumors and the lowest seen in signet ring tumors.
- Distinct biomarker features detected by IHC and sequencing suggest that TUBB3 expression may carry theranostic importance which warrants further investigation in clinical trials.
- TUBB3 expression serves as a potential resistance mechanism for taxane resistance and should be considered together with other molecular features including chromosome instabilityand expression of multidrug resistance protein when selecting for a small subgroup of taxanes responders in clinical trials.

References

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- *Melling, N., Marx, A.H, et al. "βIII-tubulin overexpression is linked to left-sided tumor localization and nuclear β-catenin expression in colorectal cancer" Cancer Treatment Communications, 2015 4, 96-102*