Differences in Molecular Profiles of Males and Females with Colorectal Cancer (CRC)

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Abstract

Background: Females (F) have a lower incidence of CRC and carry a better overall prognosis than males(M). We explored the differences in the molecular profile of CRC as an explanation for the differences in the outcome.

Methods: CRC cases submitted to Caris Life Sciences from 2015 to 2017 were analyzed. These cases were tested with next generation sequencing (NGS) of 592 genes and a panel of IHC and copy number variation assessment. Microsatellite instability (MSI) was evaluated with NGS for known MSI loci in the target regions. High Tumor mutational load (TML-H) was defined as ≥17 mutations/megabase.

Results: Data from a total of 1768 CRC tumors (F: 859; M: 909) was available for analysis. The mean age at testing was similar between the two groups (F 59 vs. M 60 years). Tumor location was unknown in more than 40% of the cases. For those with known tumor location (1056) F had a higher rate in right sided than left sided and rectal tumors (51% vs. 47% vs. 40%, p = 0.006).
Overall, F carried significantly lower frequency of mutation in APC (68% vs. 74%, p = 0.02), higher frequency of BRAF (11% vs. 6.6%, p = 0.003) and BRCA1 (2% vs. 0.6%, p = 0.007). PDL1 expression was higher in F (4.5% vs. 2.1%, p = 0.006) and MGMT expression was higher in M (63% vs. 56%, p = 0.04). There was no significant difference in the TML-H (F:6.4% vs. M:5.9%) and MSI-high (F:6.2% in vs M:4.8%).
When primary (877) and metastatic tumors (838) were investigated separately, mutations in APC was higher in M primary tumors (74% vs. 68% p = 0.03) while not different in metastatic sites. On the contrary, BRCA1 mutations were higher in the metastatic sites for F (2% vs. 0.2%, p = 0.02). PD-L1 was higher in the primary tumor of F (5.2% vs. 1.8%, p = 0.008) and PD-1 on tumor infiltrating lymphocyte in metastatic tumors in F (48% vs. 30%, p = 0.01).

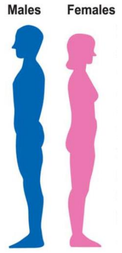
Conclusions: The profile of female patients (higher rates of PDL1 in primary and PD1 in metastatic tumors) supports a higher degree of immune evasion. The differences in the profile of metastatic vs. primary sites may be due to the differences in the mechanism of metastasis in females vs. males and may have implications for PDX models.

Methods

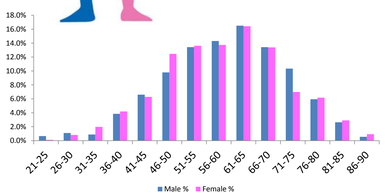
- CRC tumors profiled by Caris Life Sciences between 2015 and 2017 were de-identified and retrospectively analyzed for molecular alterations.
- Immunohistochemistry (IHC) was performed on full formalin-fixed paraffin-embedded (FFPE) sections on glass slides. Tumor cells were scored for PD-L1 and tumor infiltrating lymphocytes were scored for PD-1. Threshold used to define PD-L1 positivity was 2+, 5% and PD-1 was >1/HPF.
- NGS was performed on genomic DNA isolated from FFPE tumor samples using the NextSeq platform (Illumina, Inc., San Diego, CA). On 592 genes (SureSelect XT assay Agilent Technologies, Santa Clara, CA). All variants were detected with greater than 99% confidence based on allele frequency and amplicon coverage, with an average sequencing depth of coverage of greater than 500 and an analytic sensitivity of 5%. Prior to molecular testing, tumor enrichment was achieved by harvesting targeted tissue using manual microdissection techniques. When assessing mutation frequencies of individual genes, 'pathogenic' and 'presumed pathogenic' were counted as mutations while 'benign,' 'presumed benign,' and 'variants of unknown significance' were excluded.
- MSI was examined using over 7,000 target microsatellite loci and compared to the reference genome hg19 from the University of California, Santa Cruz Genome Browser database. TML was estimated from 592 genes (1.4 megabases [MB] sequenced per tumor) by counting all non-synonymous missense mutations found per tumor that had not been previously described as germline alterations. The threshold to define high TML was greater than or equal to 17 mutations per MB.

Results

1. Patient Characteristics



	Male		Female		Total
	N	%	N	%	N
Right-Transverse	**217**	**49%**	**228**	**51%**	**445**
Mets	59	48%	65	52%	124
Primary	156	50%	158	50%	314
Left-sided Colon	**157**	**53%**	**137**	**47%**	**294**
Mets	38	54%	33	46%	71
Primary	116	54%	98	46%	214
Colorectal cancer-Rectum	**189**	**60%**	**128**	**40%**	**317**
Mets	69	56%	54	44%	123
Primary	113	62%	68	38%	181
Unknown/Mix	**346**	**49%**	**366**	**51%**	**712**
Mets	256	49%	264	51%	520
Primary	80	48%	88	52%	168
Grand Total	**909**	**51%**	**859**	**49%**	**1768**

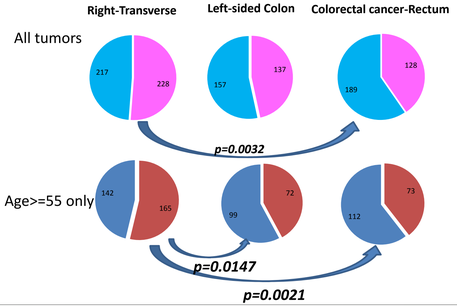


Age Group	Male N	Female N
21-25	6	1
26-30	10	7
31-35	8	17
36-40	35	36
41-45	60	54
46-50	89	107
51-55	122	117
Age <=55	**330 (36%)**	**339 (39%)**
56-60	130	118
61-65	150	141
66-70	122	115
71-75	94	60
76-80	54	53
81-85	24	25
86-90	5	8
Age >55	**579**	**520**
Total	**909**	**859**

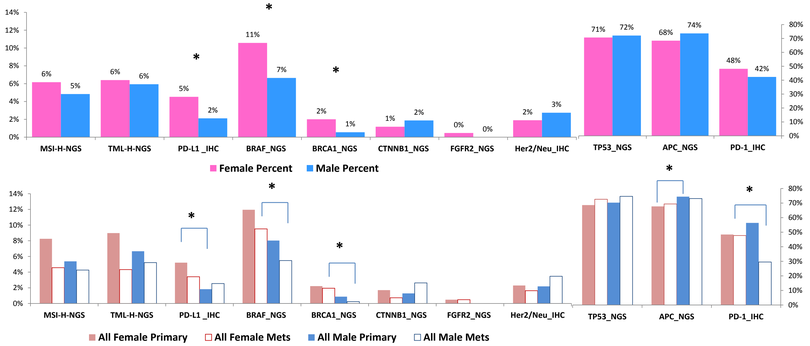
Results

2. Gender distribution is imbalanced in different tumor locations.

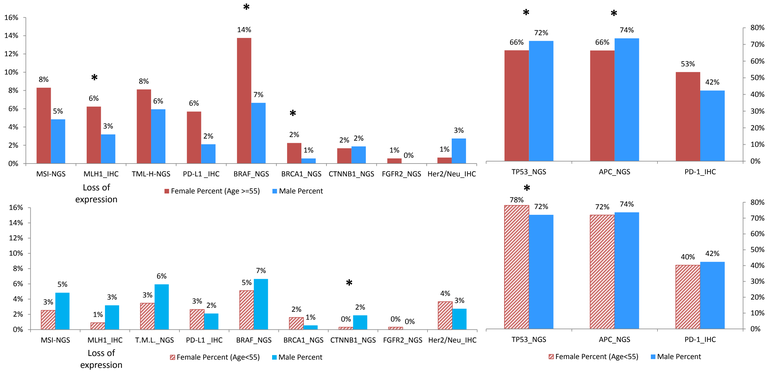
- Female gender is significantly higher in right-transverse colon compared to rectal tumors (p=0.0032) (Top)
- Among old patients, female prevalence is significantly higher in right-sided/transverse tumors compared to female prevalence in left-sided tumors (p=0.0147) and rectal tumors (p=0.0021)(bottom)



3. Comparison of selected biomarker prevalence in all female vs. male CRC tumors (Top) and in primary and metastatic tumors (bottom), respectively. A star and connective lines indicate statistical significance using Chi-Square test.



4. Comparison of selected biomarker prevalence in females older than 55 yrs vs. all male CRC tumors (Top) and in female younger than 55 yrs vs. all male CRC (Bottom) . A star indicate statistical significance using Chi-Square test.



Conclusions

- There are significant differences in the tumor location and molecular profile between females and males with colorectal cancer.
- Age, a surrogate for menopausal status, has a significant impact on the molecular profile. Immune escape (represented by PDL1 and MSI) seems to be a phenomenon of post menopausal women.
- Higher rates of right sided tumors, MSI, loss of expression of MLH1, and BRAF in older females is most likely due to methylation as a mechanism for sporadic MSI high tumors.
- Given that PDX models are used as a benchmark to assess the pre-clinical efficacy of new treatments, differences in the gender and age of the primary tumor should be considered in the selection of these models.