Ras family mutation patterns in a large cohort of CRCs

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Abstract #3599

Background: KRAS, NRAS and HRAS are functionally and structurally close proto-oncogenes with conserved sequences at important functional regions. Mutated Ras family members promote oncogenesis, cause cell proliferation and survival and confer resistance to anti-EGFR therapy. We hypothesized Ras family mutations have variable molecular and clinical features including in metastasis of CRC.

Methods: We analyzed 3677 CRCs (1992 local tumors and 1685 metastases (mets) including 620 liver, 211 lung, 181 peritoneum, 137 ovary, 19 brain, 15 bone and 4 adrenal) profiled between 2014 and 2016. NextGen sequencing was performed with Illumina MiSeq (TruSeq panel) and NextSeq (Agilent SureSelect XT assay). Microsatellite instability (MSI) was assessed.

Results: KRAS mutation occurred in 49% (1791/3640) of CRCs including exon 2 (43.1%), exon 4 (3.3%) and exon 3 (2.7%). Mutation rates were highest in bone (67%), then lung (56%), brain (53%), and no KRAS mutation was found in 4 adrenal mets. Exon 3/4 mutations accounted for 40% of brain met KRAS mutations, higher than local disease (13%, p=0.0104), lung (10%, p=0.01), liver (13%, p=0.01), ovarian (10%, p=0.002) and peritoneal mets (10%, p=0.007). KRAS mutations were more prevalent in MSI-low CRCs (846/1604, 53%) vs MSI-high CRCs (36/101, 36%, p=0.001). When specific codons are considered, KRAS G12V was the most mutually exclusive of MSI-high (1/199) while 11/138 G13D tumors (8%), 14/274 G12D (5%) tumors and 2/8 K117N CRCs were MSI-positive.

NRAS mutation occurred in 3.7% (133/3620) of CRCs, frequently in exon 3 (2.0%), then exon 2 (1.7%), but not in exon 4 or 5. Mutations were noted in adrenal (1/4, G12D), brain (1/19, G12D) and ovary (5% or 7/137), but not in bone mets (0/15). For specific codons, NRAS Q61 mutations were more prevalent vs G12/13 in CRCs of local disease (55% vs. 37%), liver (59% vs. 41%) and lung (50% vs. 33%) but were lower than codon 12/13 mutations in ovarian (43% vs 57%) and peritoneal (20% vs. 80%) mets. NRAS Q61K mutations were frequent in local disease (27% of NRAS mutations) and liver mets (30%), but were absent in lung mets (0 of 6 NRAS mutations) and rare in MSI-high CRCs (1/99, A18T).

HRAS mutation occurred in 0.3% (11/3223) CRCs, 9 in local disease, including 1 G12D mutation, and 8 variants of unknown significance in exon 2/3. Additional 2 A59T mutations occurred in a liver met and a mesenteric LN.

Conclusions: Different codon preferences are seen in RAS mutations in CRC. Ras family mutation codon preferences in metastatic CRC warrant further investigation to elucidate key drivers and personalized therapeutics.

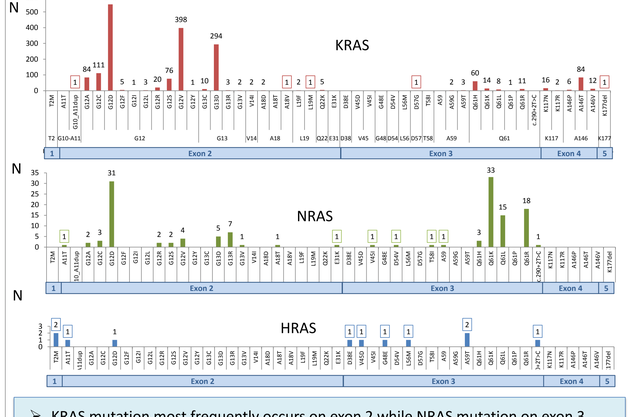
Results

Figure 1: Patient characteristics including gender, MSI status, and locations of tumor origin. Bolded percentages shows significant differences (p<0.05)

	Total	KRAS			NRAS			HRAS		
		KRAS MT (N)	KRAS WT (N)	KRAS MT (%)	NRAS MT (N)	NRAS WT (N)	NRAS MT (%)	HRAS MT (N)	HRAS WT (N)	HRAS MT (%)
Female	1770	867	880	50%	76	1672	4.3%	5	1542	0.3%
Male	1907	924	969	49%	57	1815	3.0%	6	1671	0.4%
MSI High	101	36	65	**36%**	1	98	**1.0%**	5	88	5.4%
MSI Negative	1606	846	758	**53%**	76	1517	**4.8%**	3	1445	0.2%
MSI unknown	1970	909	1026	47%	56	1872	2.9%	2	1680	0.1%
Right Colon	802	449	341	**56%**	15	781	1.9%	3	693	0.4%
Transverse Colon	68	22	45	**32%**	1	67	1.5%	0	61	0.0%
Left Colon	1108	515	585	**46%**	43	1043	4.0%	0	1108	0.0%
Colon, NOS	1699	805	878	47%	74	1596	4.4%	8	1504	0.5%
Overall	**3677**	**1791**	**1849**	**49%**	**133**	**3487**	**3.7%**	**11**	**3213**	**0.3%**

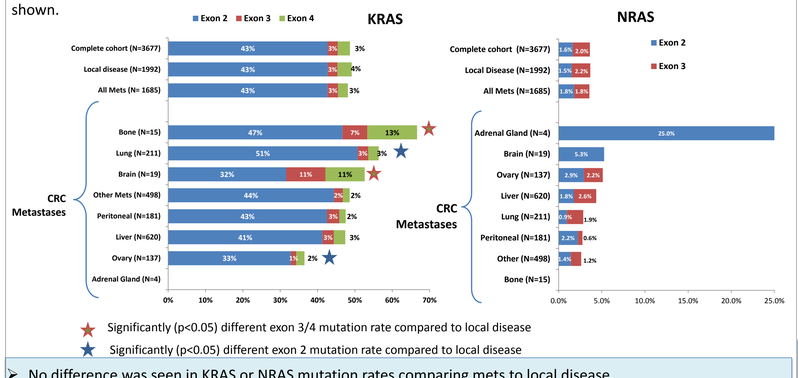
- Mutation rates of the RAS family are well-balanced between patient genders.
- KRAS mutation is significantly more frequent in MSI negative tumors (53% vs. 36%, p<0.001) while NRAS shows a trend (9% vs. 1.5%, p=0.08). An HRAS G12D mutation (see below) is seen in an MSI high tumor.
- KRAS mutation rate is the lowest in tumors originated from transverse colon and higher in left (p=0.03) and right colon (p<0.001); NRAS mutation rate is higher in the left colon than right colon (p=0.01)

Figure 2: KRAS, NRAS and HRAS mutations seen in a large cohort of CRC tumors. Boxed numbers represent mutant variants of undetermined significance (VUS). All others are pathogenic or presumed pathogenic variants .



- KRAS mutation most frequently occurs on exon 2 while NRAS mutation on exon 3.
- Exon 4 mutations are seen in KRAS, but not in NRAS or HRAS.
- HRAS mutation is overall rare in CRC tumors , only 1 mutation (G12D) is categorized as pathogenic.

Figure 3: Exon distribution of KRAS and NRAS in different CRC metastases. Frequency of KRAS (Left) NRAS (Right) mutations found in CRC cohorts, including all tumors, local disease, all metastases and individual metastatic sites are shown.

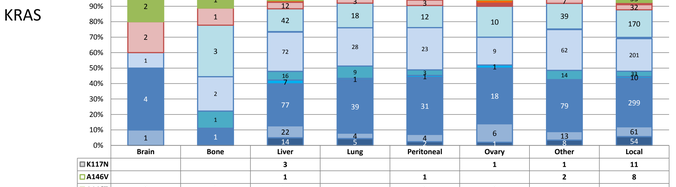


★ Significantly (p<0.05) different exon 3/4 mutation rate compared to local disease

★ Significantly (p<0.05) different exon 2 mutation rate compared to local disease

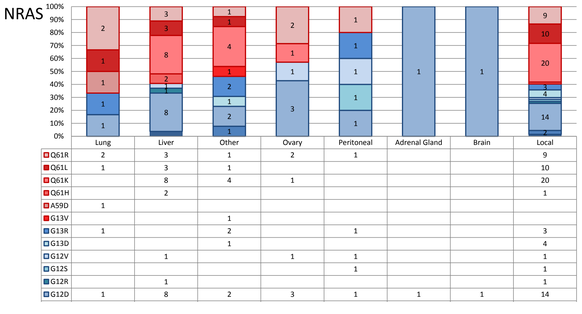
- No difference was seen in KRAS or NRAS mutation rates comparing mets to local disease.
- Bone mets carry the highest KRAS mutation frequency while no NRAS mutation is seen
  - KRAS exon 2 mutation rate is similar to local disease; exon3/4 mutation rate is significantly higher in the bone mets.
- Lung mets carry a significantly higher KRAS mutation rate than local disease:
  - Significantly higher frequency of exon 2 mutation seen in lung mets; KRAS exon3/4 mutation rates are similar
- Brain mets carry significantly higher KRAS exon 3/4 mutation than local disease, and they carry the highest NRAS mutation rate in tumor cohorts larger than 10.
- Ovarian mets carry significantly lower KRAS mutation rate compared to local disease
  - KRAS exon 2 mutation rate is significantly lower in ovarian variants; exon 3/4 mutation rate is similar when compared to local disease.
- No KRAS mutation is seen in the 4 adrenal gland metastases analyzed ; 1 NRAS mutation is seen

Figure 4: KRAS mutations in CRC metastases and local disease. Blue, red and green indicate exon 2, 3 and 4. Bars are labeled with n of each variant found.



	Brain	Bone	Liver	Lung	Peritoneal	Ovary	Other	Local
K117N			3			1	1	11
A146V			1		1		2	8
A146T	2	1	14	5	2	2	5	53
Q61R			3	1		1	2	4
Q61K					2	1	2	9
Q61H	2	1	12	3	3		7	32
G13D		3	42	18	12	10	39	170
G13C			1				1	8
G12V	1	2	72	28	23	9	62	201
G12S		1	16	9	3		14	33
G12R			7	1	1	1		10
G12D	4	1	77	39	31	18	79	299
G12C	1		22	4	4	6	13	61
G12A			14	5	2	1	8	54

Figure 5: NRAS mutations in CRC metastases and local disease. Blue and red indicate exon 2 and 3. Bars are labeled with n of each variant found.



	Lung	Liver	Other	Ovary	Peritoneal	Adrenal Gland	Brain	Local
Q61R	2	3	1	2	1			9
Q61L	1	3	1					10
Q61K		8	4	1				20
Q61H		2						1
A59D	1							
G13V			1					
G13R	1		2		1			3
G13D			1					4
G12V		1		1	1			1
G12S					1			1
G12R		1						1
G12D	1	8	2	3	1	1	1	14

- NRAS exon 3 mutations are more frequent than exon 2 mutations in lung mets, liver mets and local disease.
- The most frequent NRAS exon 2 mutation G12D is seen in all metastases while the most frequent exon3 mutation Q61K is absent in lung mets.
- Small tumor n precludes definitive conclusions and warrants further investigation.

Conclusions

- We described the mutation patterns of KRAS, NRAS and HRAS from a large clinical cohort of CRC and reported correlations with clinico-pathological features including tumor primary locations and sites of metastases.
- Consistent with previous reports, we showed that KRAS mutations predominately occur on exon 2 while NRAS on exon 3. Recently, exon 4 mutation was associated with resistance to cetuximab, and is seen in KRAS but not in NRAS or HRAS in our cohort.
- We report an emerging imbalanced RAS mutation pattern in various metastases of CRC, including
  1. High KRAS mutation in bone and brain, mainly driven by exon 3/4
  2. High KRAS mutation in lung mets, mainly driven by exon 2
  3. Low KRAS mutation rate in ovarian mets, mainly driven by exon 2
  4. Low prevalence of Q61K NRAS mutation in lung mets
- Once validated, this information will provide important insight into the genetic events driving CRC to metastasize to different organs. The potentially differential coupling with downstream effector pathways may suggest tailored treatment strategies for different CRC metastases.

References

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- El-Deiry, W.S., S. Reddy et. al. (2015) "Molecular profiling of 6,892 colorectal cancer samples suggests different possible treatment options specific to metastatic sites. "Cancer Biol Ther. 2015;16(12):1726-37.