Saudi Journal of Biological Sciences 28 (2021) 3735–3740

Contents lists available at ScienceDirect

Saudi Journal of Biological Sciences

journal homepage: www.sciencedirect.com

Original article

Analysis of RAS gene mutations in cytogenetically normal de novo acute myeloid leukemia patients reveals some novel alterations

Afia Muhammad Akram [a,*], Asma Chaudhary [a], Humera Kausar [b], Fayez Althobaiti [c], Afshan Syed Abbas [d], Zawar Hussain [a], Naz Fatima [e], Erum Zafar [a], Wajiha Asif [a], Umair Afzal [a], Zoufishan Yousaf [a], Amjad Zafar [f], Steve M. Harakeh [g], Samina Qamer [h,*]

[a] Department of Zoology, Division of Science and Technology, University of Education, Township, Lahore, Pakistan
[b] Department of Biotechnology, Kinnaird College for Women, Lahore, Pakistan
[c] Department of Biotechnology, College of Science, Taif University, P.O. Box 11099, Taif 21944, Saudi Arabia
[d] Department of Zoology, University of Education, Lower Mall Campus, Lahore, Pakistan
[e] Molecular Biology Laboratory, Department of Zoology, University of the Punjab, Lahore, Pakistan
[f] Department of Oncology, Mayo Hospital, Anarkali Bazar, Lahore, Pakistan
[g] Special Infectious Agents Unit, King Fahd Medical Research Center, King Abdulaziz University, Jeddah 21589, Saudi Arabia
[h] Department of Zoology, Government College University, Faisalabad, Pakistan

ARTICLE INFO

Article history:
Received 2 April 2021
Revised 20 April 2021
Accepted 28 April 2021
Available online 6 May 2021

Keywords:
Acute myeloid leukemia (AML)
RAS gene mutations
Kirsten rat sarcoma viral oncogene homolog (KRAS)
Neuroblastoma rat sarcoma viral oncogene homolog (NRAS)

ABSTRACT

Rat sarcoma gene (RAS) holds great importance in pathogenesis of acute myeloid leukemia (AML). The activated mutations in Neuroblastoma rat sarcoma viral oncogene homolog (NRAS) and Kirsten rat sarcoma viral oncogene homolog (KRAS) confers proliferative and survival signals, deliberating numerous effects on overall survival and progression free survival in AML patients. In this study thirty one (31) blood samples of adult newly diagnosed AML patients were collected to identify possible incidence of mutations through amplification of KRAS (exon 1 and 2) and NRAS gene (exon 1 and 2) using polymerase chain reaction (PCR). Amplicons were then subjected to sequencing and were analyzed through Geneious Prime 2019. Five of thirty one (16.12%) patients had altered sites in either NRAS or KRAS. The NRAS mutations were observed in three AML patients (N = 3, 9.67%). A novel missense mutation NRAS-I36R (239 T > G) representing a substitution of single nucleotide basepair found in NRAS exon 1 while exon 2 was detected with heterozygous mutation NRAS-E63X (318G > T) and insertion (A), resulting in frameshift of the amino acid sequence and insertion of two nucleotide basepairs (TA) in two of the patients. KRAS mutations (N = 2, 6.45%) were found in exon 1 whereas no mutations in KRAS exon 2 were detected in our patient cohort. Mutation in KRAS Exon 1, KRAS-D30N (280G > A) was observed in two patients and one of them also had a novel heterozygous mutation KRAS-L16N (240G > C). In addition there was no statistically significant association of mutRAS gene of AML patients with several prognostic markers including age, gender, karyotyping, CD34 positivity, cytogenetic abnormalities, total leukocyte count, white blood cell count and French-American-British (FAB) classification. However, the presence of mutRAS gene were strongly associated (p = 0.001) with increased percentage of bone marrow blasts. The prevalence of mutations in correlation with clinical and hematological parameter is useful for risk stratification in AML patients.

© 2021 The Author(s). Published by Elsevier B.V. on behalf of King Saud University. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

* Corresponding authors.
E-mail addresses: afiamakram@ue.edu.pk (A.M. Akram), saminabee@gmail.com (S. Qamer).

Peer review under responsibility of King Saud University.

Production and hosting by Elsevier

1. Introduction

RAS alterations are most commonly observed in AML cases ranging between 17 and 53% (Burgess et al., 2017; Yoshizato et al., 2017) and approximately 12–27% de novo AML cases (Illmer et al., 2005; Naoe & Kiyoi, 2013; Neubauer et al., 2008). RAS mutations are related to disturbance in normal hematopoiesis, disruption in signaling pathway, unregulated proliferation and

https://doi.org/10.1016/j.sjbs.2021.04.089
1319-562X/© 2021 The Author(s). Published by Elsevier B.V. on behalf of King Saud University.
This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

inadequate maturation leads to hyperproliferative developmental diseases and cancers (Metzeler et al., 2011; Sabnis et al., 2009).

Patients exhibiting wild type RAS, frequently harbor aberrations in other related genes like KIT, PDGFR and FLT3 which codes for RAS-dependent pathways. These genetic alterations activate anti-apoptotic and pro-proliferative signals crucial for leukemogenesis of myeloid cells and are termed as class I mutations (Thakore, Lehmann, McCubrey, & Terrian, 2006). AML is usually characterized by mutations related to transcription factor signaling affecting differentiation of blood forming progenitors. These are known as class 2 mutations involving fusion oncogenes formed due to balanced chromosomal translocations as t(15;17)(q22;q12) resulting in PML-RARA, t(8; 21)(q22;q22) producing RUNX1(AML1)-RUNX1T1(ETO) and (p13q22)/t(16;16)(p13;q22) also known as inv (16) (Thakore et al., 2006).

Studies on mouse models suggest that fusion genes alone cannot intensify leukemia unless accompanied by class I mutations (Chan and Gilliland, 2004). The RAS gene encodes for a family of transmembrane proteins. These proteins are involved in the regulation of signal conjugation when ligand binds to various kinds of receptors. Three active RAS genes are namely Neuroblastoma RAS (NRAS), Kirsten RAS (KRAS) and Harvey RAS (HRAS) each comprising of four exons. Mutations of RAS genes at codon 12, 13 and 61 grant natural set off of RAS proteins in GTP-bound phase (Bacher, Haferlach, Schoch, Kern, & Schnittger, 2006; Prior, Lewis, & Mattos, 2012). At first these malformations were spotted in myelodysplastic syndromes (Janssen et al., 1987); (Bos, 1989). KRAS mutations were constituting about 8–13% of AML cases. Most recurrent point mutation are occurred at different nucleotides of codons 12, 13, or 61 in KRAS (Bowen et al., 2005). The frequency of mutation KRAS exon 1 is much higher in AML patients than the KRAS exon 2. The most common base substitution was the G to A transition at codon 12 and codon 13 whereas mutation found rarely in codon 61 (Liang et al., 2006; Park et al., 2013). Mutations in the NRAS gene are frequent genetic aberrations in adult AML and are the most prominent of the RAS family; reported in approximately 11–30% of patients (Aly, El-sharnoby, & Hagag, 2011; Bacher et al., 2006; Beaupre & Kurzrock, 1999; Dunna et al., 2014; Nagarajan, 2010; Network, 2013; Pylayeva-Gupta, Grabocka, & Bar-Sagi, 2011; Shin et al., 2016; Zhou et al., 2017) and these mutations are mostly identified as G12D, G13D, G12V, Q61H, A59E, A164T (Fernández-Medarde & Santos, 2011; Hobbs, Der, & Rossman, 2016).

Mutations in RAS gene are reported in 15–30% AML patients and appear to play their role in onset of leukemia, however their prognostic role has not yet been established firmly. Some studies report worse (Boissel et al., 2006; Paquette et al., 1993) or similar (Boissel et al., 2006; Motyckova & Stone, 2010; Ritter et al., 2004; Yang et al., 2013) treatment outcome in RAS mutant patients than those who carry wild-type *RAS* gene, however others state association of RAS mutations with favorable prognosis (Neubauer et al., 1994).

High prevalence of RAS malformations was spotted in AML victims with NRAS t(3;5) and KRAS mutations inv(16) is commonly found (Valk et al., 2004). RAS malformations didn't effect prognosis remarkably in patients with normal karyotype, inv(16) and t(8;21) (Bacher et al., 2006; Bowen et al., 2005) while in case of AML with median or unfavourable karyotype, RAS mutations didn't effect prognosis. It has been found that increase in NRAS activity improve prognosis in young patients (>60 years) with infusion of high dose cytarabine showing that other activating mechanism play more key role than NRAS alterations itself (Bacher et al., 2006) whereas Patients often undergo late relapse in the presence of KRAS mutations (Tao et al., 2019; Zhou et al., 2017). In various patients KRAS mutations were not involved in guiding survival during treatment (Li, Zhang, Zhang, & Wang, 2019). Most patients received chemotherapy during treatment and expressed complete remission (CR). Usually, mutated RAS is categorized as an unfavorable indicator for complete remission and overall survival and also is linked with poor treatment outcomes. Therefore, mutation in RAS is a significant prognostic marker in the pathogenesis of AML (Verma et al., 2010). Aim of this study is to detect nucleotide alterations in NRAS and KRAS proto-oncogenes and determining their association with hematological and clinical parameters of the patients.

2. Materials and methods

2.1. Patients and ethical statement

Blood samples of 31 AML patients ranging from 16 to 45 years of age were collected by venipuncture method and were stored in EDTA tubes from Jinnah hospital, Institute of nuclear medicine and oncology (INMOL) and Mayo hospital, Lahore after obtaining informed consent from the patients. Blood samples were stored at below 20 °C within 24 h. Patient data including clinical and hematological parameters was noted on structured data forms. Patient characteristics at the time of diagnosis are given in Table 1.

2.2. Genomic DNA extraction and RAS gene amplification

Genomic DNA was extracted by using QIAamp DNA mini kit (Qiagen Cat # 51304) according to the manufacturer's protocol

Table 1
Hematological features and apparent symptoms of AML patients (n = 31) included in present study.

Clinical Measures	Features	No. of patients n = 31 (%)
Gender	Males	21 (67.7%)
	Females	10 (32.3%)
Age	<30	15 (48.4%)
	>30	16 (51.6%)
Median age	34	
Symptoms at diagnosis	Fever	25 (80.6%)
	Flu	7 (22.6%)
	Sore throat, cough	4 (12.9%)
	Spot on body	1 (3.2%)
	Swelling	2 (6.5%)
	Breathless	2 (6.5%)
	Bleeding gums	2 (6.5%)
	Pregnancy	2 (6.5%)
	Anemic	5 (16.1%)
FAB Subypes	M1	2 (6.5%)
	M2	5 (16.1%)
	M3	3 (9.6%)
	M4	10 (32.2%)
	M5	7 (22.6%)
	M6	2 (6.5%)
	M7	2 (6.5%)
Cytogenetics	Abnormal t(8:21)	2 (6.5%)
	Normal	29 (93.5%)
Liver	Enlarged	16 (51.6%)
	Normal	15 (48.4%)
Spleen	Enlarged	12 (38.7%)
	Normal	19 (61.3%)
Platelet count (109/L)	<150	25 (80.6%)
	>150	6 (19.4%)
Total leukocyte count (109/L)	<4.5	14 (45.2%)
	4.5–11	10 (32.3%)
	>11	7 (22.5%)
Hemoglobin (g/dL)	<10	24 (77.4%)
	>10	7 (22.6%)
RBCs (106/μl)	<4.2	24 (77.4%)
	>4.2	7 (22.6%)
Blast Cells (%)	<20	3(9.7%)
	>20	28(90.3%)

and stored at −20 °C till further processing. Agarose gel electrophoresis (1.2% agarose) was performed for qualitative analysis. Approximate count of DNA (using Nano Drop™) was obtained and dilutions of 1 ng/μl of each sample were formed. Integrity of genomic DNA was analysed by using housekeeping gene, Glyceraldehyde 3-phosphate dehydrogenase (GAPDH). PCR thermal cycler was used for KRAS and NRAS gene amplification according to the protocol given by (Rocquain et al., 2010). The primer sets for NRAS exon 1 (Forward 5′-AAAGTACTGTAGATGTGGCTC-3′ Reverse 5′-GTGAGAGACAGGATCAGG) exon 2 (Forward 5′-GTTATA GATGGTGAAACCTG-3′ Reverse 5′-ATACACAGAGGAAGCCTTCG-3′) and for KRAS exon 1 (Forward 5′-CGTCTGCAGTCAACTGGAAT-3′ Reverse 5′-AAAGAATGGTCCTGCACCAGTAA-3′) exon 2 (Forward 5′-GTGGCCATTTGTCCGTCATC-3′ Reverse 5′-CACCACCACTACC GATGCA-3′) were used. Working dilution of 10 pmol was prepared for each primer. For amplification and their bidirectional Sanger Sequences, each samples' extracted DNA (20 ng/μl) and four sets of primers were sent to Zixi biotechnology company Co., Ltd. China.

2.3. Sequencing data analysis

The Sequences of NRAS (exon 1 and exon 2) and KRAS (exon 1 and exon 2) of AML patients were effectively arranged and compared to the reference sequences (NCBI Reference Sequence; GenBank: NP_002515.1), (NCBI Reference Sequence: NM_002425), (NCBI Reference Sequence; GenBank: NM_004985) and (NCBI Reference Sequence; GenBank: NC_000012.12.) respectively for the detection of RAS gene mutations or any mismatching sites using Geneious Prime 2019. A control sequence was also compared to the reference sequences of each axon to determine any discrepant sites. Samples showing existence of mutations were reverse sequenced to confirm mismatching base pairs or any other alterations.

2.4. Statistical analysis

Chi square test of association-Fischer's exact test (IBM SPSS Statistics Data Editor Version 20) was used to assess possible association of hematological or clinical parameters with the existence of RAS gene mutations in patients of AML. The p value < 0.05 was considered significant, statistically.

3. Results

RAS gene was successfully amplified for our cohort (31), all AML patients were newly diagnosed. All patients were alive during the period of observation except one patient (MA17). One AML patient (INM11) had history of MDS (Myelodysplastic syndrome) which progressed to secondary acute myeloid leukemia (sAML). In our patient cohort, mean blast cell percentage was 39%. Higher percentage of blast cells has been reported to be significantly associated with occurrence of mutations.

3.1. Nras gene mutations

NRAS mutations were detected positive in three AML patients (9.6%) for both exon 1 and 2. Two patients (INM2 & INM7) were cytogenetically normal while one (INM5) was cytogenetically abnormal having translocation t(8;21).

The patient (INM-2) was observed to harbor amino acid substitution I36R (239 T > G) in NRAS exon 1 (Table 2). Two AML patients (INM5 and INM7) manifested insertion of one nucleotide base pair adenine (A) at position 319 of codon 63 following a heterozygous mutation at position 318 (G → T) in NRAS exon 2, causing frameshift in the subsequent amino acid sequence (Fig. 1).

The normal reading frame reads the translation product, GLU (glutamic acid; GAG) however, because of the insertion of one nucleotide base pair adenine (A), the amino acid sequence changes from GAG → GAA which also codes for glutamic acid (Table 2) but it causes frameshift effect leading to pre-mature stop codon formation and truncated protein (Fig. 1). Another insertion of one nucleotide basepair thymine (T) was detected at position 336 of codon 68 in one patient (INM7) ceasing the translation due to formation of stop codon instead of Arg (arginine). However, in another patient (INM5), further insertion of two nucleotide basepairs thymine and adenine (TA) occurred at position 336 and 337. This insertion of two nucleotide basepair causes the formation of premature stop codon resulting in the termination of translation (Fig. 1).

3.2. Kras gene mutations

KRAS exon 1 mutations were observed in two patients (6.45%) while no aberration was detected in exon 2. Both mutant

Table 2
Mutant Patients of AML with identified mutations and their resulting amino acid changes.

Patient ID	Gender	Age	FAB Subtype	Nucleotide Change/Insertion	Amino-Acid change	Novel/Reported
NRAS exon 1						
INM2	Female	30	M2	239 T → G	Ile36Arg	Novel
NRAS exon 2						
INM5	Female	24	M2	318 G → T Insertion of Adenine (A) at position 319	Glu63stop codon Glu63Glu	Novel
INM7	Male	21	M1/M2	Insertion of Thymine and Adenine at position 336 and 337 318 G → T Adenine (A) at position 319 Thymine at position 336	Arg68stop codon Glu 63stop codon Glu63Glu Arg68stop codon	Novel
KRAS exon 1						
MA17	Female	22	M4	237C → A 280G → A	Gly15Gly Asp30Asn	(Kuwabara, Tanabe, Warashina, Xiong, Tani, Taira, and Asano, 2001)
MA20	Female	22	M4	237C → A 280G → A 240G → C	Gly15Gly Asp30Asn Lys16Asn	(Kuwabara, Tanabe, Warashina, Xiong, Tani, Taira, and Asano, 2001) Novel

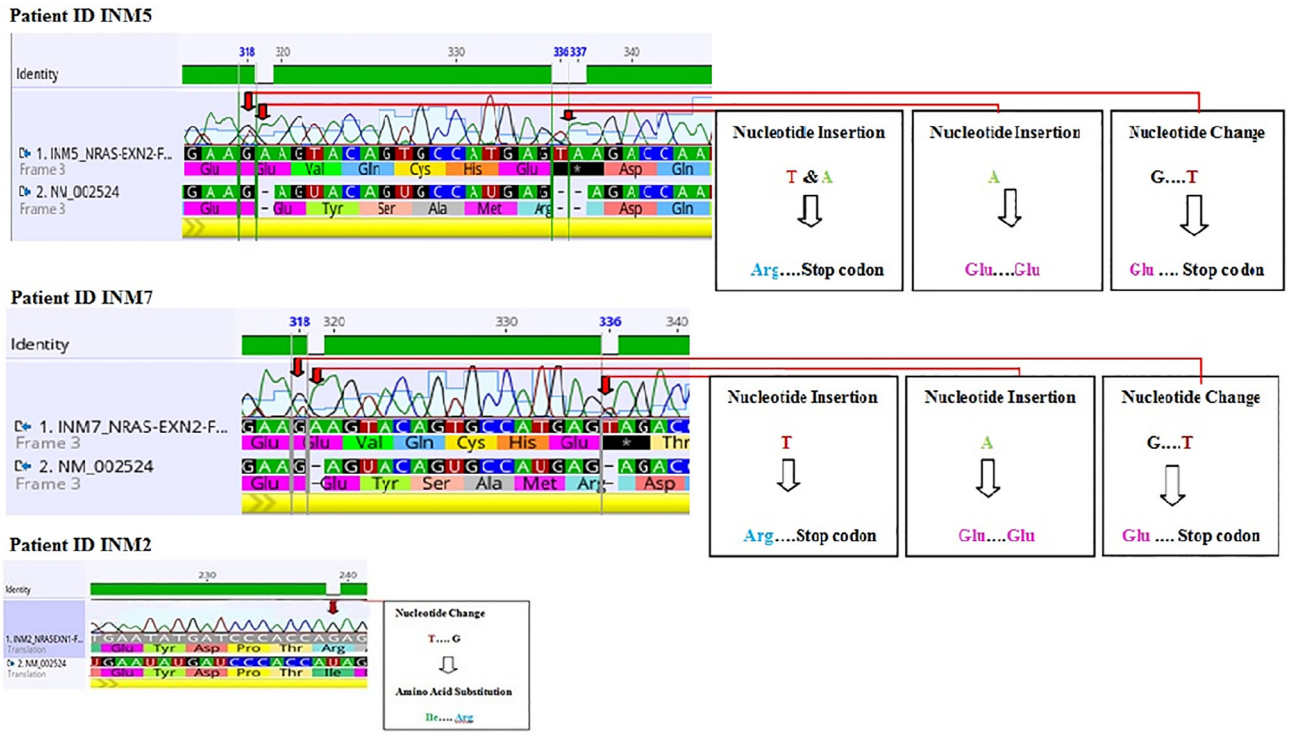

Fig. 1. Electropherogram of three AML patients detected positive for NRAS gene aberrations. Hetrozygous mutation along with insertion of one or two nucleotide basepair was found in two AML patients INM5 and INM7 while a substitution 239 T > G was detected in INM2. Nucleotide change or insertion and amino acid substitution is mentioned in the relevant boxes. (INM2 & INM5 = females, INM7 = male).

Fig. 2. Electropherogram of two AML patients detected positive for KRAS gene aberrations. Hetrozygous mutation at position 237 and 280 was found in two AML patients MA17 and MA20 for KRAS exon 1. Nucleotide change and amino acid change is mentioned in the relevant boxes.

individuals were normal, cytogenetically and harbored silent and heterozygous mutations. These patients (MA17 and MA20) had a nucleotide substitution cytosine to adenine (C → A) at position 237 forming the same amino acid Glycine (GGC → GGA) as the normal reading frame represents a silent mutation (Gly15Gly) (Table 2). Same patients (MA17 and MA20) held a heterozygous mutation at position 280 from Guanine to adenine(G → A) which resulted in formation of asparagine AAC instead of Aspartic acid GAC i.e., (Asp30Asn) (Fig. 2).

4. Discussion

We found more females than males harboring mutations in RAS gene and a significant association was observed between gender and occurrence of mutations, which is in contradiction to other reported studies (Bolufer et al., 2007; Hossain & Xie, 2015). Higher blast percentage was somewhat similar to previous investigations (Asif & Hassan, 2013; Dicker et al., 2010; Jeong et al., 2013).

Earlier studies have frequently reported point mutations 35G > A (Gly12Asp) and 38G > A (Gly13Asp) associated with inv (16)/t(16;16), while 313A > G (Gln61Arg) is also often observed in AML patients(Bacher et al., 2006; Barlas et al., 2020; Farr, Saiki, Erlich, McCormick, & Marshall, 1988). The mutation 35G > A induces the sensitive sites such as P-loop, switch I and II in NRAS gene causing flux in these regions impeding the binding of GTP (Radich et al., 1990). AML patients mutated with 35G > A have more opening of GTP binding pocket (Chen et al., 2013). The mutation disrupts intrinsic and RAS-GAP–mediated GTP hydrolysis, leading to constitutive activation (Herrmann, 2003; Moodie, Willumsen, Weber, & Wolfman, 1993) and increased affinity of RAS to the direct effectors, RAF-1 (McGrath, Capon, Goeddel,

& Levinson, 1984) and PI3K, regulatory links of mitogen-activated protein kinase (MAPK) pathway (Bivona, 2019; Marcus & Mattos, 2015; Sjölander, Yamamoto, Huber, & Lapetina, 1991). According to a number of studies, patients having NRAS gene mutations show overall poor prognosis (Bacher et al., 2006; Coghlan, Morley, Matthews, & Bishop, 1994; Neubauer et al., 2008) or no clinical prognosis at all including complete remission rate, overall survival, disease free survival and relapse-free survival (Bacher et al., 2006; Bowen et al., 2005; Coghlan et al., 1994; Paulsson et al., 2008; Radich et al., 1990; Stirewalt et al., 2001). However, in many studies patients positive for mutations in NRAS gene are indicated to have positive prognosis (Coghlan et al., 1994; Kiyoi et al., 1999; Meyer et al., 2009; Neubauer et al., 2008). Some researchers believe that there exists no survival difference among NRAS mutant and non-mutant patients of AML (Nakagawa et al., 1992). Previous studies support the fact that NRAS gene mutation can be an important prognostic biomarker in relation to poor survival of children with AML (Liu et al., 2019).

As evident from similar studies, these activating mutations in KRAS gene have potential effect on cellular physiology. It can be anticipated that aggressive disease patterns may exist in mutant individuals. However, sometimes RAS activity can induce loss of cell division and growth along with enhanced apoptotic signaling (Ahmad, Gawish, Al Azizi, & Elhefni, 2011). Literature supports the existence of somatic mutations in KRAS gene due to receptor tyrosine kinases (Ghukasyan et al., 2020). Another study reported up-regulation of KRAS gene expression and mutations in 8% of the AML population. This may be attributed to old age, increased number of WBCs, and elevted platelet number (Zhou et al., 2017). Studies have also reported RAS mutations as most frequent mutations representing 54% in inv(16) and 30% in t(8;21) in patients with AML (Awada et al., 2019). Some reports looked for an association between RAS gene mutations and patient karyotype, blast cell load and FAB subtypes, but the results were not remarkable.

5. Conclusion

The findings of this study show that not only substitutions at codon 12, 13 and 61 can be responsible for development of acute myeloid leukemia but nucleotide insertions along with hetrogeniety and point mutations other than these codons contribute as well to the onset of disease. A heterozygous mutation was found in one patient with t(8;21) while other patients were cytogenetically normal. This suggests that these aberrations can be detected in both cytogenetically normal as well as abnormal AML patients. Genotype demarcated by NRAS gene is associated with poor treatment outcome in AML patients. For further understanding of association of identified mutations with disease pattern and prognosis, extensive studies are needed, as these alterations may present a target for molecular therapy.

Declaration of Competing Interest

The authors do not have any conflicts of interest. The authors alone are responsible for the content and writing of the paper.

Acknowledgements

We acknowledge the Oncology department, Mayo hospital Lahore and Department of Oncology, Jinnah hospital, Lahore for this collaborative work. This study was funded by Higher education commission of Pakistan (HEC), grant number 9727/Punjab/NRPU/R&D/HEC/2017. Laboratory facilities provided by department of Zoology, University of Education, Township Lahore are highly acknowledged. This work was also funded by Taif University Researches Supporting Project number (TURSP-2020/222), Taif University, Taif, Saudi Arabia.

References

Ahmad, E.I., Gawish, H.H., Al Azizi, N.M., Elhefni, A.M., 2011. The prognostic impact of K-RAS mutations in adult acute myeloid leukemia patients treated with high-dose cytarabine. OncoTargets Ther. 4, 115–121. https://doi.org/10.2147/OTT.S12602.

Aly, R.M., El-sharnoby, M.R., Hagag, A.A., 2011. Prognostic significance of NRAS gene mutations in children with acute myelogenous leukemia. Mediterranean J. Hematol. Infect. Dis. 3 (1).

Asif, N., Hassan, K., 2013. Acute myeloid leukemia amongst adults. J. Islamabad Med. Dental College 2, 58–63.

Awada, H., Kishtagari, A., Kuzmanovic, T., Durrani, J., Kerr, C.M., Meggendorfer, M., Haferlach, T., 2019. Impact and Outcomes of RAS gene Mutations in Core Binding Factor Acute Myeloid Leukemia. American Society of Hematology Washington, DC.

Bacher, U., Haferlach, T., Schoch, C., Kern, W., Schnittger, S., 2006. Implications of NRAS mutations in AML: a study of 2502 patients. Blood 107 (10), 3847–3853.

Barlas, S., Robert, H.M., Mahmood, R., Mahmood, A., Khurshid, A., Khan, S.A., 2020. Frequency of nras and kras genes in newly diagnosed acute myeloid leukemia patients. Pakistan Armed Forces Med. J. 70 (2), 447–452.

Beaupre, D.M., Kurzrock, R., 1999. RAS and leukemia: from basic mechanisms to gene-directed therapy. J. Clin. Oncol. 17 (3). 1071-1071.

Bivona, T.G., 2019. Dampening oncogenic RAS signaling. Science 363 (6433), 1280–1281.

Boissel, N., Leroy, H., Brethon, B., Philippe, N., De Botton, S., Auvrignon, A., Hermine, O., 2006. Incidence and prognostic impact of c-Kit, FLT3, and Ras gene mutations in core binding factor acute myeloid leukemia (CBF-AML). Leukemia 20 (6), 965–970.

Bolufer, P., Collado, M., Barragán, E., Cervera, J., Calasanz, M.-J., Colomer, D., et al., 2007. The potential effect of gender in combination with common genetic polymorphisms of drug-metabolizing enzymes on the risk of developing acute leukemia. Haematologica 92 (3), 308–314.

Bos, J.L., 1989. Ras oncogenes in human cancer: a review. Cancer Res. 49 (17), 4682–4689.

Bowen, D.T., Frew, M.E., Hills, R., Gale, R.E., Wheatley, K., Groves, M.J., Burnett, A.K., 2005. RAS mutation in acute myeloid leukemia is associated with distinct cytogenetic subgroups but does not influence outcome in patients younger than 60 years. Blood 106 (6), 2113–2119.

Burgess, M.R., Hwang, E., Mroue, R., Bielski, C.M., Wandler, A.M., Huang, B.J., et al., 2017. KRAS allelic imbalance enhances fitness and modulates MAP kinase dependence in cancer. Cell 168 (5), 817–829. e815.

Chan, I.T., Gilliland, D.G., 2004. Oncogenic K-ras in mouse models of myeloproliferative disease and acute myeloid leukemia. Cell Cycle 3 (5), 534–535.

Chen, C.-C., Er, T.-K., Liu, Y.-Y., Hwang, J.-K., Barrio, M.J., Rodrigo, M., Herreros-Villanueva, M., 2013. Computational analysis of KRAS mutations: implications for different effects on the KRAS p. PLoS ONE 8 (2), e55793.

Coghlan, D., Morley, A., Matthews, J., Bishop, J., 1994. The incidence and prognostic significance of mutations in codon 13 of the N-ras gene in acute myeloid leukemia. Leukemia 8 (10), 1682.

Dicker, F., Haferlach, C., Sundermann, J., Wendland, N., Weiss, T., Kern, W., Schnittger, S., 2010. Mutation analysis for RUNX1, MLL-PTD, FLT3-ITD, NPM1 and NRAS in 269 patients with MDS or secondary AmL. Leukemia 24 (8), 1528–1532.

Dunna, N.R., Vuree, S., Anuradha, C., Sailaja, K., Surekha, D., Digumarti, R.R., et al., 2014. NRAS mutations in de novo acute leukemia: prevalence and clinical significance.

Farr, C.J., Saiki, R.K., Erlich, H.A., McCormick, F., Marshall, C.J., 1988. Analysis of RAS gene mutations in acute myeloid leukemia by polymerase chain reaction and oligonucleotide probes. Proc. Natl. Acad. Sci. 85 (5), 1629–1633.

Fernández-Medarde, A., Santos, E., 2011. Ras in cancer and developmental diseases. Genes Cancer 2 (3), 344–358.

Ghukasyan, L., Krasnov, G., Muravenko, O., Ikonnikova, A., Yurasov, R., Baidun, L., Nasedkina, T., 2020. Driver mutations in acute myeloid leukemia with inversion of chromosome 16. Mol. Biol. 54 (3), 341–348.

Herrmann, C., 2003. Ras–effector interactions: after one decade. Curr. Opin. Struct. Biol. 13 (1), 122–129.

Hobbs, G.A., Der, C.J., Rossman, K.L., 2016. RAS isoforms and mutations in cancer at a glance. J. Cell Sci. 129 (7), 1287–1292.

Hossain, M.J., Xie, L., 2015. Sex disparity in childhood and young adult acute myeloid leukemia (AML) survival: evidence from US population data. Cancer Epidemiol. 39 (6), 892–900.

Illmer, T., Thiede, C., Fredersdorf, A., Stadler, S., Neubauer, A., Ehninger, G., Schaich, M., 2005. Activation of the RAS pathway is predictive for a chemosensitive phenotype of acute myelogenous leukemia blasts. Clin. Cancer Res. 11 (9), 3217–3224.

Janssen, J., Steenvoorden, A., Lyons, J., Anger, B., Böhlke, J.U., Bos, J.L., Bartram, C.R., 1987. RAS gene mutations in acute and chronic myelocytic leukemias, chronic myeloproliferative disorders, and myelodysplastic syndromes. Proc. Natl. Acad. Sci. 84 (24), 9228–9232.

Jeong, J.H., Park, S.H., Park, M.J., Kim, M.J., Kim, K.H., Park, P.W., Hong, J., 2013. N-ras mutation detection by pyrosequencing in adult patients with acute myeloid leukemia at a single institution. Ann. Lab. Med. 33 (3), 159–166.
Kiyoi, H., Naoe, T., Nakano, Y., Yokota, S., Minami, S., Miyawaki, S., Shimazaki, C., 1999. Prognostic implication of FLT3 and N-RAS gene mutations in acute myeloid leukemia. Blood J. Am. Soc. Hematol. 93 (9), 3074–3080.
Li, S., Zhang, P., Zhang, Y., Wang, L., 2019. The role of ras oncogene mutations in acute myeloid leukemia patients: a meta-analysis based on 2502 cases. Adv. Appl. Physiol. 3 (2), 44.
Liang, D.C., Shih, L.Y., Fu, J.F., Li, H.Y., Wang, H.I., Hung, I.J., Liu, H.C., 2006. K-Ras mutations and N-Ras mutations in childhood acute leukemias with or without mixed-lineage leukemia gene rearrangements. Cancer 106 (4), 950–956.
Liu, X., Ye, Q., Zhao, X.-P., Zhang, P.-B., Li, S., Li, R.-Q., Zhao, X.-L., 2019. RAS mutations in acute myeloid leukaemia patients: a review and meta-analysis. Clin. Chim. Acta 489, 254–260.
Marcus, K., Mattos, C., 2015. Direct attack on RAS: intramolecular communication and mutation-specific effects: AACR.
McGrath, J.P., Capon, D.J., Goeddel, D.V., Levinson, A.D., 1984. Comparative biochemical properties of normal and activated human ras p21 protein. Nature 310 (5979), 644–649.
Metzeler, K.H., Maharry, K., Radmacher, M.D., Mrózek, K., Margeson, D., Becker, H., Whitman, S.P., 2011. TET2 mutations improve the new European LeukemiaNet risk classification of acute myeloid leukemia: a cancer and Leukemia Group B study. J. Clin. Oncol. 29 (10), 1373.
Meyer, M., Rübsamen, D., Slany, R., Illmer, T., Stabla, K., Roth, P., Neubauer, A., 2009. Oncogenic RAS enables DNA damage-and p53-dependent differentiation of acute myeloid leukemia cells in response to chemotherapy. PLoS ONE 4 (11), e7768.
Moodie, S.A., Willumsen, B.M., Weber, M.J., Wolfman, A., 1993. Complexes of Ras. GTP with Raf-1 and mitogen-activated protein kinase kinase. Science 260 (5114), 1658–1661.
Motyckova, G., Stone, R.M., 2010. The role of molecular tests in acute myelogenous leukemia treatment decisions. Curr. Hematol. Malignancy Rep. 5 (2), 109–117.
Nagarajan, L., 2010. Acute Myelogenous Leukemia. Springer.
Nakagawa, T., Saitoh, S., Imoto, S., Itoh, M., Tsutsumi, M., Hikiji, K., et al., 1992. Multiple point mutation of N-ras and K-ras oncogenes in myelodysplastic syndrome and acute myelogenous leukemia. Oncology 49 (2), 114–122. https://doi.org/10.1159/000227023.
Naoe, T., Kiyoi, H., 2013. Gene mutations of acute myeloid leukemia in the genome era. Int. J. Hematol. 97 (2), 165–174.
Network, C.G.A.R., 2013. Genomic and epigenomic landscapes of adult de novo acute myeloid leukemia. N. Engl. J. Med. 368 (22), 2059–2074.
Neubauer, A., Dodge, R., George, S., Davey, F., Silver, R., Schiffer, C., et al., 1994. Prognostic importance of mutations in the ras proto-oncogenes in de novo acute myeloid leukemia.
Neubauer, A., Maharry, K., Mrózek, K., Thiede, C., Marcucci, G., Paschka, P., Bloomfield, C.D., 2008. Patients with acute myeloid leukemia and RAS mutations benefit most from postremission high-dose cytarabine: a Cancer and Leukemia Group B study. J. Clin. Oncol. 26 (28), 4603.
Paquette, R.L., Landaw, E.M., Pierre, R., Kahan, J., Lubbert, M., Lazcano, O., et al., 1993. N-ras mutations are associated with poor prognosis and increased risk of leukemia in myelodysplastic syndrome.
Park, M.-J., Park, S.-H., Park, P.-W., Seo, Y.-H., Kim, K.-H., Jeong, J.-H., Park, J., 2013. Frequency of KRAS mutations in adult Korean patients with acute myeloid leukemia. Int. J. Hematol. 98 (5), 549–557.
Paulsson, K., Horvat, A., Strömbeck, B., Nilsson, F., Heldrup, J., Behrendtz, M., Johansson, B., 2008. Mutations of FLT3, NRAS, KRAS, and PTPN11 are frequent and possibly mutually exclusive in high hyperdiploid childhood acute lymphoblastic leukemia. Genes Chromosom. Cancer 47 (1), 26–33.
Prior, I.A., Lewis, P.D., Mattos, C., 2012. A comprehensive survey of Ras mutations in cancer. Cancer Res. 72 (10), 2457–2467.
Pylayeva-Gupta, Y., Grabocka, E., Bar-Sagi, D., 2011. RAS oncogenes: weaving a tumorigenic web. Nat. Rev. Cancer 11 (11), 761.
Radich, J.P., Kopecky, K.J., Willman, C.L., Weick, J., Head, D., Appelbaum, F., Collins, S., 1990. N-ras mutations in adult de novo acute myelogenous leukemia: prevalence and clinical significance.
Ritter, M., Kim, T.D., Lisske, P., Thiede, C., Schaich, M., Neubauer, A., 2004. Prognostic significance of N-RAS and K-RAS mutations in 232 patients with acute myeloid leukemia. Haematologica 89 (11), 1397–1399.
Rocquain, J., Carbuccia, N., Trouplin, V., Raynaud, S., Murati, A., Nezri, M., Birnbaum, D., 2010. Combined mutations of asxl1, cbl, flt3, idh1, idh2, jak2, kras, npm1, nras, runx1, tet2 and wt1 genes in myelodysplastic syndromes and acute myeloid leukemias. BMC Cancer 10 (1), 1–7.
Sabnis, A.J., Cheung, L.S., Dail, M., Kang, H.C., Santaguida, M., Hermiston, M.L., Braun, B.S., 2009. Oncogenic Kras initiates leukemia in hematopoietic stem cells. PLoS Biol. 7 (3), e1000059.
Shin, S.-Y., Lee, S.-T., Kim, H.-J., Cho, E.H., Kim, J.-W., Park, S., Kim, S.-H., 2016. Mutation profiling of 19 candidate genes in acute myeloid leukemia suggests significance of DNMT3A mutations. Oncotarget 7 (34), 54825.
Sjölander, A., Yamamoto, K., Huber, B.E., Lapetina, E.G., 1991. Association of p21ras with phosphatidylinositol 3-kinase. Proc. Natl. Acad. Sci. 88 (18), 7908–7912.
Stirewalt, D.L., Kopecky, K.J., Meshinchi, S., Appelbaum, F.R., Slovak, M.L., Willman, C.L., Radich, J.P., 2001. FLT3, RAS, and TP53 mutations in elderly patients with acute myeloid leukemia. Blood J. Am. Soc. Hematol. 97 (11), 3589–3595.
Tao, C., Wang, Q., Ho, D., Polat, T., Nallan, L., Soon-Shiong, P., 2019. Substituted indol-5-ol derivatives and their therapeutic applications: Google Patents.
Thakore, C.U., Lehmann, B.D., McCubrey, J.A., Terrian, D.M., 2006. Intracellular signaling in cancer. Rev. Cell Biol. Mole. Med.
Valk, P., Bowen, D.T., Frew, M.E., Goodeve, A.C., Lowenberg, B., Reilly, J.T., 2004. Second hit mutations in the RTK/RAS signaling pathway in acute myeloid leukemia with inv (16). Haematologica 89 (1). 106-106.
Verma, D., Kantarjian, H., Faderl, S., O'Brien, S., Pierce, S., Vu, K., Ravandi, F., 2010. Late relapses in acute myeloid leukemia: analysis of characteristics and outcome. Leukemia Lymphoma 51 (5), 778–782.
Yang, X., Qian, J., Sun, A., Lin, J., Xiao, G., Yin, J., Wu, D., 2013. RAS mutation analysis in a large cohort of Chinese patients with acute myeloid leukemia. Clin. Biochem. 46 (7–8), 579–583.
Yoshizato, T., Nannya, Y., Atsuta, Y., Shiozawa, Y., Iijima-Yamashita, Y., Yoshida, K., Sato, Y., 2017. Genetic abnormalities in myelodysplasia and secondary acute myeloid leukemia: impact on outcome of stem cell transplantation. Blood 129 (17), 2347–2358.
Zhou, J.-D., Yao, D.-M., Li, X.-X., Zhang, T.-J., Zhang, W., Ma, J.-C., Qian, J., 2017. KRAS overexpression independent of RAS mutations confers an adverse prognosis in cytogenetically normal acute myeloid leukemia. Oncotarget 8 (39), 66087.