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

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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 frame-shift 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.

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

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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 1 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 of 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 (Jiang 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 intensify leukemia unless accompanied by class I mutations (Thakore et al., 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).

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

| 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 Subtypes**  |          |                           |
| M1                | 2 (6.5%)   |                        |
| M2                | 2 (6.5%)   |                        |
| 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)  | 3 (9.7%)   |                        |
| Normal            | 28 (90.3%) |                        |
| **Liver**         |          |                           |
| Enlarged          | 16 (51.6%) |                        |
| Normal            | 15 (48.4%) |                        |
| **Spleen**        |          |                           |
| Enlarged          | 12 (38.7%) |                        |
| Normal            | 9 (29.0%)  |                        |
| **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, Glycer-aldehyde 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'-AAAGTACTGATGATGGCTC-3' Reverse 5'-GTTGAGACAACCTGAAGG) exon 2 (Forward 5'-GTGAGATGGTGAAACCTG-3') and for KRAS exon 1 (Forward 5'-CGTCTGACTCACTGGAAAAT-3' Reverse 5'-AAAGAATGGTCCTGCACCAGTAA-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_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 (NCBI Reference Sequence; GenBank: NC_000012.12.) for the sequences (NCBI Reference Sequence; GenBank: NM_002425), (NCBI Reference Sequence: NM_004985) and (NCBI Reference Sequence: NM_002425), respectively for KRAS exon 1 (Forward 5'-GATGCA-3' Reverse 5'-AAAGTACTGTAGATGTGGCTC-3') and for KRAS exon 2 (Forward 5'-AAAGAATGGTCCTGCACCAGTAA-3' Reverse 5'-CAACACACTACCAGTACA-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.

2.4. 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 cyogenetically normal while one (INM5) was cyogenetically 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

| Patient ID | Gender | Age | FAB Subtype | Nucleotide Change/Insertion | Amino-Acid change | Novel/Reported |
|------------|--------|-----|-------------|-----------------------------|------------------|---------------|
| NRAS exon 1 | Female | 30 | M2 | 239 T → G | Ile36Arg | Novel |
| NRAS exon 2 | Female | 24 | M2 | 318 G → T Insertion of Adenine (A) at position 319 | Glu63stop codon Glu63Glu | Novel |
| INM5 | Male | 21 | M1/M2 | Insertion of Thymine and Adenine at position 336 and 337 | Arg68stop codon Glu63stop codon Glu63Glu | Novel |
| INM7 | Male | 21 | M1/M2 | Thymine at position 336 | Arg68stop codon | |
| KRAS exon 1 | Female | 22 | M4 | 237C → A 280G → A | Gly15Gly Asp30Asn | (Kuwabara, Tanabe, Warashina, Xiong, Tani, Taira, and Asano, 2001) |
| MA17 | Female | 22 | M4 | 237C → A 280G → A | Gly15Gly Asp30Asn | (Kuwabara, Tanabe, Warashina, Xiong, Tani, Taira, and Asano, 2001) |
| MA20 | Female | 22 | M4 | 240G → C | Lys16Asn | Novel |
individuals were normal, cytogenetically and harbored silent and heterozygous mutations. These patients (MA17 and MA20) had a nucleotide substitution cytosine to adenine (C \rightarrow A) at position 237 forming the same amino acid Glycine (GGC \rightarrow 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 \rightarrow 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, & Bispop, 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 (Chukasyan 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 elevated platelet number (Zhou et al., 2017). Studies have also reported RAS mutations as most frequent mutations of Zoology, University of Education, Township Lahore are highly.

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 heterogeneity 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 discriminate 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.

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 heterogeneity 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 discriminate 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.

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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. Oncol Targets Ther. 4, 115–121. https://doi.org/10.2147/OTT.S121602.

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., Kishitagarai, A., Kuzmanovic, T., Durranji, I., 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., Khushrid, A., Khan, S.A., 2020. Frequency of ras 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. Damping oncogenic ras signaling. Science 363 (6433), 1280–1281.

Bois, J.L., 1989. Ras oncoproteins 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, J.M., 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 MAPK pathway dependence in cancer. Cell 168 (5), 817–829, e815.

Chan, L.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-Villarroya, 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. Leukaemia 20 (6), 965–970.

Bolognese, P., Collado, M., Barrajón, E., Cervera, J., Calasanz, M.J., Colonner, 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.

Burrill, 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 MAPK pathway dependence in cancer. Cell 168 (5), 817–829, e815.

Illmer, T., Thiede, C., Fredersdorf, A., Stadler, S., Neubauer, A., Ehninger, G., Schach, M., 2005. Activation of the RAS pathway is predictive for a chemosensitive myeloproliferative disease, and myelodysplastic syndromes. Proc. Natl. Acad. Sci. 84 (24), 9228–9232.
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