Cytogenomic profiling and clinical correlation of 21q22 amplification in acute myeloid leukemia reveal distinct cytogenomic features and poor outcomes

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Background

21q22 amplification is a rare cytogenetic aberration in acute myeloid leukemia (AML). So far, the cytogenomic and molecular features and clinical correlation of 21q22 amplification in AML have not been well-characterized. Here, we describe a case series of three AML patients with amplified 21q22 identified by fluorescence *in situ* hybridization (FISH) using a *RUNX1* probe. Two of these patients presented with therapy-related AML (t-AML) secondary to chemotherapy, while the third had *de novo* AML. There was one case each of FAB M0, M1 and M4. Morphologic evidence of dysplasia was identified in both t-AML cases. Phenotypic abnormalities of the myeloblasts were frequently observed. Extra copies of 21q22 were present on chromosome 21 and at least one other chromosome in two cases. Two showed a highly complex karyotype. Microarray analysis of 21q22 amplification in one case demonstrated alternating levels of high copy number gain split within the *RUNX1* locus at 21q22, a pattern distinct from the iAMP21 profile reported in B-cell precursor acute lymphoblastic leukemia (B-ALL). The same patient also had mutated *TP53*. Two patients died at 1.5 and 11 months post-treatment, while the third elected palliative care and died within 2 weeks. Our results provide further evidence that 21q22 amplification in AML is associated with complex karyotypes, *TP53* aberrations, and poor outcomes. Furthermore, we demonstrate that 21q22 amplification is not always intrachromosomally localized to chromosome 21 and could be a result of structural aberrations involving 21q22 and other chromosomes.

Objectives

- Characterize the features of 21q22 amplification in AML using multiple cytogenomic methods
- Compare 21q22 amplification to previously reported cases
- Contrast 21q22 amplification in AML to iAMP21 in B-ALL

Case Selection & Methods

- 3 AML patients with extra copies of *RUNX1* FISH signal were incidentally detected by routine AML FISH screening for *RUNX1-RUNX1T1* t(8;21)(q21.3;q22) fusion
- G-banded karyotype, interphase FISH, metaphase FISH, microarray, and molecular analysis were performed to characterize cytogenomic & molecular features
- 2/3 patients presented with therapy-related AML and all 3 patients were adults

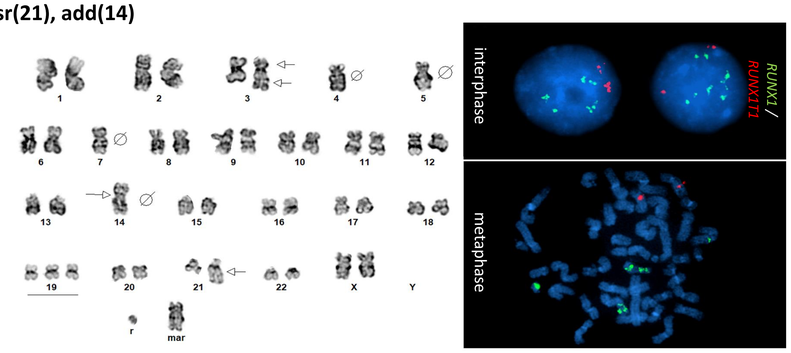
Pt	Age /Sex	Diagnosis	Prior Malignancy	Treatment and Outcome
1	77/F	t-AML	Follicular Lymphoma	Venetoclax+ Azacitadine. Died within 1.5 months
2	26/M	t-AML	Hodgkin Lymphoma	Palliative care. Died within 2 weeks
3	32/F	AML	None	Idarubicin + cytarabine, double cord transplant. Died 11 months

Results

- All patients carried amplifications of 21q22, ranging from 4-15 copies of RUNX1 signal detected during interphase FISH
- 2/3 patients had subsequent metaphase FISH performed and *RUNX1* signal was localized to multiple chromosomes in addition to chromosome 21, suggesting structural rearrangements involving the amplification

Patient 1

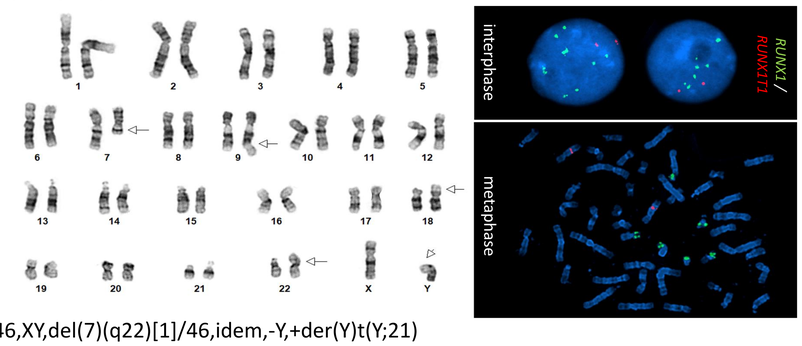
RUNX1 FISH copy number and localization: *RUNX1*x5-10, present on **marker, hsr(21), add(14)**



45,XX,der(3)inv(3)(p25q25)add(3)(q11.2),-5,-7,+12,-14,add(14)(p11.2),+19,hsr(21)(q22)[3]/45,sl,-4,-12,+r,+mar[15]/46,sdl1,+8[2]

Patient 2

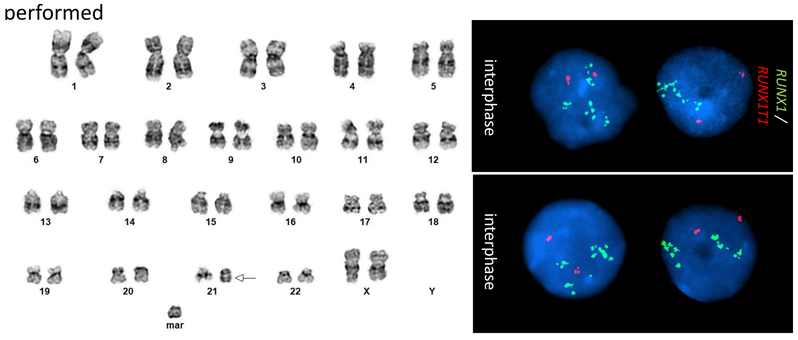
RUNX1 FISH copy number and localization: *RUNX1*x4-15, present on **Yp, 5q, 9q, 18q, 22p**



46,XY,del(7)(q22)[1]/46,idem,-Y,+der(Y)t(Y;21)(p11.3;q22.1),der(5)t(5;21)(q35;q22.1)[5]/46,idem,-Y,+der(Y)t(Y;21)(p11.2;q22.1)dup(21)(q22.1q22.3),der(9)t(9;21)(q34;q22.1)dup(21)(q22.1q22.3),der(18)t (18;21)(p11.3;q22.1)[14]

Patient 3

RUNX1 FISH copy number and localization: *RUNX1*x10, metaphase FISH not performed

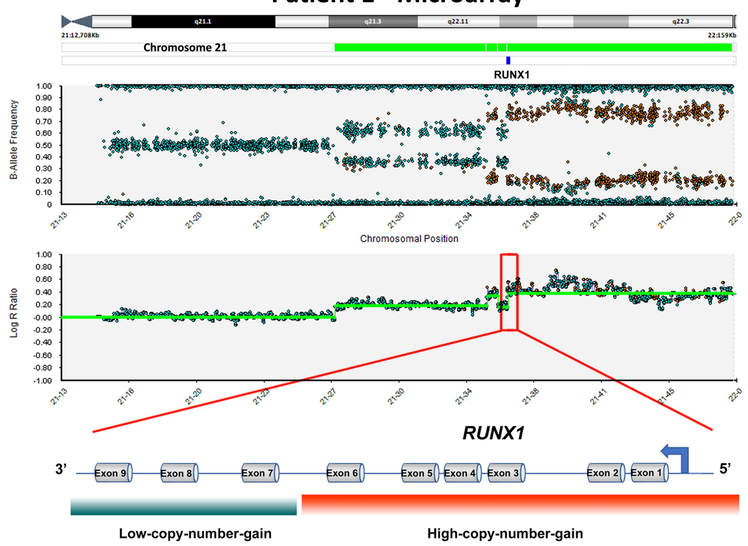


46,XX,?r(21)dup(21)(q11.2q22)[6]/47,sl,+mar[10]/46 ,XX[4]

Results

- Patient 1 was analyzed by SNP microarray and found to carry alternating segments of low and high copy number gain on 21q22
- The *RUNX1* gene was not uniformly amplified, similar to Nguyen 2019, suggesting it is unlikely to be a key driver gene within the amplification

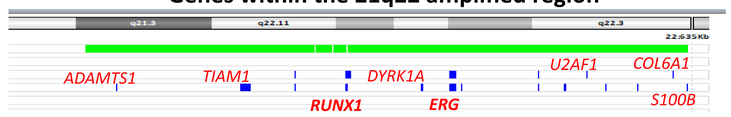
Patient 1 - Microarray



Cytogenetic band	Genomic coordinates	Size	Copy Number
21q21.3 - 21q22.11	Chr21:27,129,093 - 35,134,557	8.0 Mb	~3
21q22.11 - 21q22.11	Chr21:35,138,326 - 35,725,729	587.4 Kb	~3-6
21q22.11 - 21q22.12	Chr21:35,726,226 - 36,228,360	502.1 Kb	~3
21q22.12 - 21q22.3	Chr21:36,230,819 - 48,100,155	11.9 Mb	~3-6

- What is the driver within the 21q22 amplified region? Previous studies have identified *ERG* amplification in AML patients (Weber 2016) – may be important target?

Genes within the 21q22 amplified region



Conclusions

- 21q22 amplification was identified in 0.2% of AML patients in CGL from 2011 – 2021. The prevalence may be underestimated since all cases were incidentally detected by *RUNX1* FISH
- 21q22 amplification is not always confined to a single chromosome (unlike iAMP21 in B-ALL) and can translocate to multiple chromosomes. This may represent a different entity than seen in B-ALL
- Patients carrying this aberration may present with t-AML or *de novo* AML, usually exhibit a complex karyotype, and have poor outcomes

References

- Nguyen D, Li Y, Safah H, Brown TC (2019) RUNX1 deletion/amplification in therapy-related acute myeloid leukemia: A case report and review of the literature. Cancer Genet 238:37-43.
- Weber S, Haferlach C, Jeromin S, Nadarajah N, Dicker F, Noel L, Zenger M, Alpermann T, Kern W, Haferlach T, Schnittger S (2016) Gain of chromosome 21 or amplification of chromosome arm 21q is one mechanism for increased ERG expression in acute myeloid leukemia. Genes Chromosomes Cancer 55 (2):148-157