

Deletions of chromosome 5 in myeloid disorders

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The first description of terminal or interstitial deletion of chromosome 5 was reported by Van den Berghe in 1974 in three patients with refractory anemia (1). This description represented the second structural chromosome change to be discovered in human hematologic malignancies. In 1975 Sokal termed the 5q- syndrome as a distinct hematological disorder associated with refractory macrocytic anemia and thrombocytosis (2). Currently, the 5q- syndrome is applied to patients with myelodysplastic syndrome (MDS) showing three hematological features: macrocytic anemia, normal or high platelet count and megakaryocytic hypolobulation in most megakaryocytes. There is a preponderance of females with these characteristics. However, the criteria that have been applied to the diagnosis of 5q- syndrome have varied. Suffice to say, that these patients have favorable prognosis because they have low rate of transformation to acute leukemia and preservation of peripheral blood counts with low incidence of ineffective episodes. Association of del(5q) with treatment was first reported by Rowley in 1981 (3) who showed that 88% of patients with lymphoma who were treated with chemo-radiation or the combination developed myelodysplasia (MDS) or acute myeloid leukemia (AML). The most recent updated review was presented by M.M. Le Beau in 1996 at the educational session of the American Society of Hematology. The experience of University of Chicago showed that 71% of patients with treatment-related MDS and AML have abnormalities of chromosome 5. We reported in 1992 that although rare, deletion of chromosome 5 may be occasionally detected in patients with ALL expressing biphenotypic lineage: myeloid, TdT and early progenitor phenotype CD34 (4).

Recently Van den Berge combined data on 1,432 patients with del(5q) and showed that AML, specifically M1/M2 type, most frequently has, only del(5q) as the sole abnormality, while AML types M2, M4, and M6 have del(5q) together with other rearrangements (5). The frequency of del(5q) in myeloid disorders is different: in de novo AML del(5q) is not as common as in therapy-related AML. In contrast, about 30% of patients with de novo MDS have del(5q) and in these patients it is associated with favorable prognosis. Our experience at the Mount Sinai Medical Center from 1986 to May 1999, confirmed that del(5q) is found in 25% of patients with MDS and in 3.8% (22/576) of patients with de novo AML at the time of diagnosis.

There appears to be a great heterogeneity in breakpoints. At least 42 deletions have been described. Both interstitial and terminal breakpoints for just about every chromosome band has been reported. The heterogeneity of some breakpoints may be attributed to the difficulty in determining the exact breakpoints when chromosome morphology is of suboptimal quality. Two chromosomal regions that are most frequently deleted are 5q11 or 5q12 most frequently found in AML, and 5q31 most frequently deleted in MDS. Furthermore, 5q31 was deleted in all subgroups of MDS which had only a 5q31 rearrangement. In contrast, in AML, with or without the additional chromosomal rearrangements, the bands 5q21, 5q22 and 5q23 were deleted more often than 5q31. From the literature, it appears that the presence of del(5)(q31) seems to be associated exclusively with a low tendency towards cytogenetic progression.

For this reason, investigators in the field have speculated for years that a tumor suppressor gene or genes may be located in 5q22-23 or 5q31. There are a

number of genes that are mapped to the distal region of the long arms of chromosome 5. Of note are *Il-2*, *Il-4*, *Il-5*, *Il-9* which are all mapped to 5q23-31. On the 5q31 band are: early growth response-1 gene (*EGR-1*), interferon regulatory factor 1 (*IRF1*), granulocyte-macrophage colony stimulating factor (*GM-CSF*), fibroblasts growth factor (*FGF1*) and macrophage colony stimulating factor-1 receptor (*CSF1R*). To delineate the commonly deleted segment at the molecular level, Le Beau et al performed fluorescence in situ hybridization (FISH) with a panel of probes to metaphase cells from patients who had either a proximal or distal breakpoint within 5q31. They postulated that a tumor suppressor gene, originally in a 3-4 Mb region that has been narrowed to a 1-1.5 Mb region. The only known genes within this interval are *EGR1* and *CDC25C* genes. Analysis of leukemia cells with *del(5q)* did not reveal mutations in either gene.

In 1995, we published a mapping study of a gene named *PURA* (7). This gene was mapped to chromosome 5, band region q31. *PURA* is a sequence specific single stranded DNA protein with the following characteristics: it binds an element at eukaryotic origin of DNA replication; it binds with high affinity to the single-stranded DNA PUR element (CGN)_n; it binds in vivo to the hypophosphorylated form of Rb and is released at G1/S phase of cell cycle; and it co-localizes in the nucleus with Cyclin ACCK2. Very recently, in 1999, we also published the localization of histone deacetylase 3 gene (*HDAC3*) to 5q31.3 (8). Histone deacetylases are regulatory proteins involved in both the repression and activation of transcription. Human *HDAC3* is a single-copy gene spanning a region of at least 13 kb.

Localization of *PURA* and *HDAC3* to the 5q31 region provided the rationale for an interphase FISH study to determine if these loci may be used as markers for *del(5)(q31)* in patients with MDS, therapy-related AML, de novo AML and other myeloid disorders. Further, a metaphase FISH study in the patients may provide correct cytogenetic assignments of 5q31 breakpoints. We studied 25 patients with *PURA* and 11 patients using *HDAC3*. Concordant results between conventional cytogenetics and interphase FISH was obtained in 22 of 25 patients (88%) with *PURA* and in 9 of 11 patients using *HDAC3*. The reasons for discordant results in these patients was inaccurate

breakpoint assignment and multiple rearrangements of chromosome 5 which could not be determined by conventional cytogenetics. Interphase FISH with *PURA* and *HDAC3* on 30 patients with MDS and a normal karyotype did not reveal any cryptic 5q31 translocations. No mutations were detected in *PURA* of three patients with MDS and *del(5q)*. Fine mapping revealed the localization of *PURA* on 5q31 downstream from *EGR-1* and linked to D5S1867 (9). These studies demonstrated that interphase and metaphase FISH with *PURA* and *HDAC3* is a highly sensitive method not only for detection of *del(5)(q31)* but also for more accurate breakpoint assignments than conventional cytogenetics. Deletion of either *PURA* or *HDAC3* may cause dysregulation in transcriptional events critical for the pathogenesis of myeloid disorders.

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