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Mismatch repair defects among italian cases of secondary acute leukemia and myelodysplastic syndrome

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Purpose: The frequency of secondary acute leukemia (sAL) and myelodysplastic syndrome (sMDS) is increasing as a consequence of successful therapy for primary malignancies. Estimates of the proportion of sAL cases displaying microsatellite instability (MSI), the hallmark of DNA mismatch repair deficiency, vary widely. We are examining MSI and hMLH1 promoter methylation in a panel of Italian cases in order to investigate: the frequency of mismatch repair defects, the mechanism by which repair genes are inactivated and which therapeutic regimes are particularly associated with mismatch repair inactivation Methods: DNA was extracted from mononuclear bone marow cells collected at diagnosis from 23 patients (18 sAML, 2 sMDS, 3 sALL) most of whom had received previous therapy for Hodgkin or non-Hodgkin lymphoma or breast carcinoma. Microsatellites BAT26, BAT25, D2S123, D17S250, D18S61 were analyzed by PCR. Samples with alterations at BAT26 were considered to be MSI+. Where normal DNA was available, instability was examined at the other loci. Methylation of the hMLH1 promoter was examined by PCR following digestion with either HpaII or MspI. Results: 14/22 DNA samples from which BAT26 could be amplified were MSI+. In the 8 cases for which normal DNA was available, instability was confirmed at additional loci. One case in which BAT 26 was not amplifiable was unstable at 2 other loci (BAT25 and D2S123). Evidence of hMLH1 promoter methylation was obtained for two samples, both of which were MSI+. The hMLH1 promoter was unmethylated in three other MSI+ cases. All (4/4) secondary malignancies from Hodgkin or Non-Hodgkin lymphoma patients treated with a methylating agent (procarbazine or dacarbazine) were MSI+. Conclusions: Inactivation of MMR is common among sAL and sMDS which may be MSI+ in > 60% of cases. Methylation of the hMLH1 promoter does occur but is not the only mechanism of MMR inactivation. MSI+ sAL is particularly – but not exclusively – associated with the use of methylating agents.

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Cell cycle arrest and induction of apoptosis by novel Cdk inhibitor MCS-5A is associated with p16Ink4A up-regulation in Human promyelocytic leukemia cell

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Purpose: MCS-5A, novel Cdk inhibitor, has been reported that it has exerted cell cycle arrest action and apoptotic effect to the human promyelocytic leukemias cell. The purpose of this study is to verify these effects of MCS-5A on HL-60 cells and to clarify the action of mechanism on MCS-5A-inducing apoptosis.

Methods: HL-60 cells were evaluated for antiproliferative effect and apoptosis using cell viability test, protein kinase assay, DNA fragmentation, Flow cytometry assay and electromicroscopic examination. To clarify the action of mechanism, we also performed immunoblot assay for cell cycle and apoptosis proteins. To determine the possible of apoptosis to pcDNA-p16 mediated cytotoxicity, we tansfected pcDNA-p16 transiently and performed TUNEL assay on A549 cell, human lung cancer cell, which has homozygous deletion of p16.

Results: We investigated the involvement of cell cycle regulatory events during MCS-5A mediated apoptosis in HL-60 cells. The treatment of HL-60 cells with MCS-5A(3uM, 12hrs) resulted in inhibition of the phosphorylation of Rb protein, a critical step for G1/S transition. The kinase activities of Cdk4, Cdc2 were inhibited in HL-60 cells treated with MCS-5A(IC50 values of 8.8 and 9.6μM, respectively). Furthermore, MCS-5A increases the level of Cdk inhibitor p16. MCS-5A promoted binding of p16 to Cdk4. The induction of apoptosis by MCS-5A is associated with p16 up-regulation. Transient transfection of A549 cell with pcDNA-p16 resulted in a rightward shift of the mean fluorescene intensity when compared to the baseline fluorescence following tansfection with pcDNA vector. MCS-5A can induce apoptosis through different pathway of caspase activation with caspase-8 and caspase-9 playing a pivotal role. Caspase-8 can also activate the pro-apoptotic Bcl-2 family member Bid through proteolytic cleavage. The activation of caspase-9 in MCS-5A treated HL-60 cells is likely to occur via the caspase-8-Bid-mitochondria pathway which leads to cytochrome c release, followed by cleavage of caspase-9. MCS-5A exerted antiproliferation of HL-60 through the induction of apoptosis which is mediated by p16 and caspase-3 pathway, not by mitochondrial pathway.

Conclusions: These results indicate that MCS-5A exerts cell cycle arrest and apoptosis inducing activity in HL-60 cells and might have a potent activity as a new concept anticancer agent in human leukemias and that p16 is capable of mediating apoptosis in human cancer cells

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Deletion of chromosome 15 represents a rare but recurrent chromosomal abnormality in myeloid malignancies

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Chromosomal abnormalities characterize biological and prognostic subgroups of acute leukemias and point to genes relevant for malignant transformation and disease progression. We report on three cases with myeloid disorders cytogenetically characterized by a deletion of the long arm of chromosome 15 occurring as the sole cytogenetic aberration. The deletions were defined as del(15)(q12q21) (two cases) and del(15)(q11q21) (one case), respectively. Two cases were diagnosed with an acute myeloid leukemia (AML) with dysplastic features classified as AML-M6 and AML-M4 according to the FAB classification. The third case had a chronic myelomonocytic leukemia. In two cases, the aberration was found at the time of primary diagnosis, whereas the third case showed the del(15) during relapse of the leukemia. Both cases with acute leukemia did not adequately respond to intensive chemotherapeutic treatment and died 13 and 11 months, respectively, after primary diagnosis. Cytogenetic analysis was supplemented by fluorescence in situ hybridization using a chromosome 15 specific whole chromosome painting probe and a cosmid probe hybridizing to the PML gene located on 15q22. Hereby, a cryptic translocation involving chromosome 15 could be excluded. Moreover, we could show that the breakpoint occurred proximal to the PML gene, which was retained in both cases analyzed.

Our finding and the data of the 7 previously published cases with an isolated del(15) indicate that (1) del(15) represents a rare but recurrent abnormality in myeloid hemopathies, (2) del(15) occurs frequently in disorders with myelodysplastic or myeloproliferative features and may therefore affect early hematopoietic progenitor cells and (3) del(15) may occur during disease progression and is often associated with an unfavourable prognosis.

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Genetical alterations occur in the atypical bronchial epithelium accompanying interstitial pulmonary fibrosis (IPF)

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Background and purpose: High incidence of lung cancer is reported in patients with IPF. We investigated the genetic alterations in the atypical bronchial epithelia (ABE) accompanying benign lung diseases including IPF.

Materials and methods: We performed LOH (loss of heterozygosity) analysis on the short arm of chromosome 3 (3p) and 9 (9p) and evaluated by utilizing polymerase chain reaction(PCR) and direct sequencing analysis of K-ras oncogene. A total of 215 transbronchial biopsy specimens diagnosed as ABE were used for the study. These specimens were obtained from 74 benign lung diseases and 29 concurrent lung cancers.

Results: LOH frequency at 3p21.3 was 32%. The comparison between the frequency of LOH at the 3p14.2 (FHIT) in smokers and that in non-smokers was statistically significant (34% vs. 3.9%, $p < 0.0001$). In the ABE lesions obtained from benign lung diseases, 16 specimens demonstrated LOH at the 9p22 (IFNA) locus. In these 16 specimens, 14 (88%) of them were obtained from IPF. All of these 16 specimens demonstrated LOH at one or two chromosomal 3p loci. Mutations of K-ras codon 12/13 were found two cases of IPF patients.

Conclusion: These results suggest that genetical alterations occasionally occur in the lesions with ABE particularly in IPF patients. Since high incidence of lung cancer is reported in patients with IPF, the presence