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DNA Repair in Acute Myeloid Leukemia and Myelodysplastic Syndromes

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1. Introduction

Acute myeloid leukemia (AML) comprises approximately 25% of all leukemias in adults in the western world. It is a clonal disorder caused by uncontrolled proliferation and accumulation of myeloid progenitor cells in the bone marrow with impaired differentiation, leading ultimately to hematopoietic failure.

Myelodysplastic syndrome (MDS) is characterized by persistent pancytopenia, dysplastic hematopoesis in bone marrow, increase in blast cell number and high risk of progression to AML. MDS is a disease of elderly, which makes treatment difficult (Estey 2008). The median age of AML patients is also high and is estimated to be 66 to 70 years. Standard therapeutic strategies in MDS and AML depend on various factors, of which age, comorbidities and performance status are most important. Treatment modalities vary from the best supportive care through low dose chemotherapy to intensive dose chemotherapy and allogeneic bone marrow transplantation (Robak T, Wierzbowska A 2009). Untreated MDS and AML carry extremely poor prognosis with high mortality. The search for new drugs in AML and MDS is stimulated by the significant progress in the understanding of the biology of both diseases.

Abnormal myeloid cells usually carry chromosomal anomalies, including translocations, deletions, and allelic loss. Typical cytogenetic changes seen in AML are balanced translocations such as t(8,21), t(15,17) which result in formation of a fusion gene. MDS is characterized by deletions of fragments or whole chromosomes hence loss of genetic information. DNA methylation dysregulation is one of the postulated mechanisms of leukemia development and progression. Hypermethylation of DNA generally results in a decreased expression of tumor suppressor genes and defective cell cycle control and is a hallmark of MDS and AML. Epigenetic changes augment genetic alterations occurring in cancer cells and promote tumor progression. Several sequential events in the genom are required to create a leukemic clone. Defects of DNA repair are the key mechanism of development and progression of myeloid leukemias. Most of the AML cases originate de novo, but around 10 to 20% of patients have previous exposure to myelotoxic substances. Preceding anticancer treatment or exposure to chemical toxins may result in severe damage to DNA and in case of defective DNA repair mechanisms lead to secondary AML. DNA repair mechanisms may influence not only the risk of leukemia development, but also its refractoriness to treatment.

Several major pathways of DNA repair exist: Homologous Recombination (HR), Non Homologous End Joining (NHEJ), Base Excision Repair (BER), Nucleotide Excision Repair

(NER), Mismatch Repair (MMR), Translesion DNA synthesis (TLS) (D'Andrea 2010, Shrivastav, de Haro et al 2008). HR and NHEJ are responsible for repair of DNA double strand breaks (DSB), caused mainly by ionizing radiation, free radicals, and chemical toxins including cytostatics. DSBs comprise DNA lesions most detrimental for cell survival. The other DNA repair mechanisms deal with single strand breaks and the presence of improper base or alkyl adducts in the DNA. A complementary strand is used as the repair template. Different DNA repair processes overlap in their function and usually one problem can be repaired in 2 different ways. In normal cells all of DNA repair pathways are active and balanced. In the case of irreversible DNA damage, cells are directed to apoptosis. When the damage is moderate and repair processes inadequate the cells accumulate dangerous mutations and genomic instability occurs. This is the first step of neoplastic transformation. The defective function of one of the DNA repair pathways often results in overexpression of the other one. Increased processes of DNA repair may lead to resistance to cytostatics and radiotherapy (Pallis, Karamouzis 2010). The role of DNA damage and repair processes in pathogenesis and treatment of cancer was first noted in patients with inherited syndromes with defective DNA repair mechanisms such as Fanconi anemia. Much information comes also from neoplastic disorders induced by factors known to damage DNA, such as ionizing radiation or cytostatic therapy.

2. Secondary AML and MDS

Secondary AML and MDS are most common therapy related neoplasms. Two main types of treatment-related AML exist depending on type of cytostatics administered. Secondary leukemia due to topoisomerase II inhibitors such as podophilotoxins and antracycline antibiotics, used in variety of solid tumors, occurs usually after short period of time (1 year) following chemotherapy. Characteristic features include chromosome 11q23 anomalies, total or partial deletion of chromosome 7 and certain balanced translocations such as t (8,21) or t(15,17). The mechanism of development of that type of treatment related AML is not clearly understood, but defects in DSB repair are thought to be a key mechanism. Antracycline antibiotics intercalate into DNA and stabilize DNA-topoisomerase II complex and promote DSB formation. The cytostatics induce DSB at sites concerning hematopoetic transcription factors such as MLL, AML1/CBFB, RARA and additionally decrease rejoining of generated DSB. HR and NHEJ proper function is therefore crucial for restoring genome integrity. Otherwise, accumulating mutations lead to malignant transformation, thus patients with impaired DNA repair may be predisposed to chemotherapy induced leukemia (Guillem, Tormo 2008).

The other type of secondary leukemia is concerned with the previous use of alkylating agents. AML is diagnosed usually 5-7 years after chemotherapy and often follows MDS phase. Cytogenetic events common in this type of secondary leukemia are total or partial deletion of chromosome 5 and 7. Alkylating agents produce damage to the DNA forming monoadducts and diadducts. The process may result in interstrand and intrastrand cross links, producing single and double stand breaks. The damage caused by alkylating agents activates various DNA repair pathways. Monoadducts are usually repaired by NER and BER. Diadducts are managed by NER and HR. MMR by its influence on HR also takes part in repair processes induced in response to alkylator treatment. Cells with defective MMR function show an increased expression of RAD51, one of components of HR, and an increased microsatellite instability (Worillow, Allan et al. 2006).

3. HR defects in AML and MDS

HR, together with NHEJ, is responsible for repair of DSB, the most important DNA lesion. DSB are produced naturally, especially during a normal programmed genom rearrangement and after the exposure to DNA toxic agents. HR requires homologous sequence to that of the broken end to start the repair process and in human cells deals mainly with DBS located within the replication forks. HR is more accurate than NHEJ.

Impaired HR is a hallmark of Fanconi anemia (FA), a rare inherited disorder. Different 13 FA proteins work together in HR DNA repair pathway and a defect of 1 of those proteins results in similar clinical phenotype: short stature, skeletal defects, bone marrow failure and hypersensitivity to DNA damaging agents such as mitomycin C. The patients are at high risk of developing AML and solid tumors, mainly gynecological. Diagnostic test for FA is based on detection of defective DNA repair: examination of chromosome breakage after exposure to mitomycin C or diepoxybutane. Chromosomal aberrations typical for FA patients with AML are also found in de novo AML, thus the knowledge based on this genetic disorder help us to understand the biology of AML. DNA repair defects typical for Fanconi anemia put those patients at high risk of AML and solid tumor development, especially breast, ovarian and pancreatic cancer. Defects in HR activity similar to those detected in AML patients with Fanconi anemia, were seen in AML secondary to previous anticancer treatment. Their occurrence in patients with de novo AML is rare.

Single nucleotide G/C polymorphism in position 135 in gene encoding main protein active in HR pathway, RAD51, is correlated with AML predisposition (Seedhouse , Foulkner 2004). A polymorphism at codon 241 of another HR gene, XRCC3, results in Thr to Met substitution. Both polymorphisms were known previously to increase solid cancer susceptibility. The presence of both RAD51-135 C and XRCC3-241 Met protein variants increased the risk of secondary and primary AML development, 8 and 4 fold respectively.

Observations made in patients with solid tumors with defective HR suggest increased sensitivity of cancer cells to cis-platin and to DNA repair inhibitors such as PARP or ATM inhibitors. So far such strategies are still at preclinical phase.

4. NHEJ defects in AML and MDS

NHEJ, together with HR, constitutes main pathways to repair DNA double strand breaks. NHEJ rejoins broken fragments with little requirement for homology and is extremely important in avoiding radiation toxicity. Translocation and mutations at the junction of the broken ends happen, that is why NHEJ is responsible for tumorigenic processes. Proper and balanced functions of NHEJ and HR are necessary to maintain genom integrity.

Chromosomal instability in myeloid neoplasms results from deregulated NHEJ and inadequate DSB repair. The rate of NHEJ in leukemic blasts was 2-7 fold higher than in normal hematopoietic cells *in vitro* and resulted in increased misrepair , especially large deletions (Gaymes, Mufti et al. 2002).

The number of double strand breaks is increased by the excessive reactive oxygen species (ROS) production. Certain genetic changes such as FMS - like tyrosine kinase 3 (FLT3) mutation in AML and RAS mutations in MDS are responsible for induction of ROS generation and lead to genomic instability. FLT3 mutations occur in half of the patients with AML. Activating mutation is seen in 30-35% of cases while internal tandem duplication (FLT3 /ITD) in 20-25% of AML cases. The presence of FLT3/ITD carries extremely bad

prognosis in AML and is correlated with increased ROS generation and increased double strand brakes in DNA (Sallmyr, Fan et al. 2008). In FLT3/ITD-containing cells NHEJ is defective, which results in accumulating aberrant DNA structures (Seedhouse, Hunter et al. 2006). Thus the cells depend on another DNA repair pathway. Upregulation of RAD51, the main component of HR pathway, was noted in patients with FLT3/ITD, defective in NHEJ, and could be partially responsible for resistance to chemotherapy (Seedhouse, Hunter et al 2006).

Increased ROS generation and accumulation of double strand brakes are seen also in patients with N- RAS mutation in MDS and AML. In addition to enhanced survival and proliferation of the cells, N-RAS mutation results in ineffective NHEJ leading to DNA instability (Rassool, Gaymes et al. 2007).

5. BER in AML and MDS

BER pathway is concerned with the removal of the base changed by alkylation, oxidation or ionizing radiation. BER also takes part in the single strand breaks repair. A defective base is detected and removed and the gap is filled by DNA polymerase, then the fragments are joined by XRCC1 /ligase III complex.

Malfunction of BER pathway may contribute to cancer development. Data concerning polymorphism of XRCC1, one of the genes belonging to the BER pathway, in solid tumors are not conclusive, some studies show predisposition to the cancer in a wild type allele, the other do not. In AML studies, polymorphism of this gene (XRCC1 Arg399Gln) protects from development of the disease, especially of a secondary type. Patients with treatment related AML are more likely to have the wild –type of XRCC1 399Arg allele (Seedhouse, Bainton 2002).

Important components of BER pathway are the poly(ADP-ribose) polymerases (PARP) family containing 18 members. Those proteins allow for access to DNA repair enzymes in the case of single strand DNA breaks. After recruitment of PARP proteins the strands are cut, repaired and rejoined. PARP1 is the best studied protein in the PARPs family. Inhibition of PARP1 results in conversion of single to double strand breaks increasing the need for HR repair. Cancer cells with defective HR processes switch to PARP mediated BER mechanisms. Hence, PARP1 inhibitors are extremely active in tumors deficient in HR pathway and thus converting DNA repair to BER. Such treatment could be alternative for selected AML patients with impaired HR (Gaymes, Shall 2009).

6. NER in AML and MDS

NER is able to eliminate a wide variety of DNA damage, for example, long adducts such as pyrimidine dimers or crosslinks caused by chemotherapy. It is responsible also for the elimination of DNA damage resulting from UV radiation and chemical substances. NER is necessary both in maintaining global genom integrity and in repair of actively transcribed genes. Long sequences of improper oligonucleotides can be excised in a multi-step process requiring helicases and nucleases followed by ligation of the repaired DNA fragments.

Genes belonging to XP (xeroderma pigmentosum) group B and D encode helicases, enzymes responsible for unwinding DNA prior to transcription or NER. XPB protein is bound and modified by p210, the product of fusion gene bcr-abl responsible for chronic myeloid leukemia. Defective DNA helicases enhance genetic instability observed in this disorder.

The role of NER in AML pathogenesis and prognosis has also been investigated. Common polymorphisms in XPD gene belonging to NER pathway are associated with the risk of AML development and the outcome of the disease. XPD Lys 751 Gln variant is an independent prognostic marker for disease free survival and overall survival in elderly patients with AML (Allan, Smith 2004). Glutamine variant has altered enzymatic function with an impaired cellular response to genotoxins and is associated with the worse outcome as compared to lysine one. Heterozygotes had an intermediate AML outcome while homozygotes in the glutamine variant had the shortest overall and disease free survival. Homozygocity for the glutamine variant was also correlated with an increased risk of developing secondary AML after chemotherapy, but not after radiotherapy. Homozygocity did not affect de novo AML incidence. Moreover, the presence of the glutamine variant of XPD gene was associated with unfavorable cytogenetic profile in AML including patients with 5q and 7 q deletions (Smith, Worrillow et al. 2007). The same single nucleotide polymorphisms of XPD gene (XPD in Lys 751 Gln) together with the polymorphism of another gene in the same family (XPC Ala499 Val) were tested recently in AML patients with normal cytogenetics (Strom, Estey et al. 2010). Each polymorphism was an independent adverse prognostic factor for overall survival. Patients with the combination of variants in both genes had a significantly shorter overall survival (median 12 months) than carriers of wild type genes (median 44 months p=0.001).

7. MMR in AML and MDS

Processes of MMR play an important role in maintaining genome stability by detecting and repairing small insertions, deletions or misplaced bases which may occur during replication. Defects of MMR result in an increased rate of spontaneous mutations. Multiple replication errors occur in repetitive DNA sequences leading to microsatellite instability. Inadequate MMR may increase the risk of solid tumors and myeloid neoplasms (Ben-Yehuda, Krichevsky et al. 1996; Seedhouse, Das-Gupta et al. 2003). Microsatellite instability was observed in secondary leukemia and in elderly patients with AML (Das-Gupta, Seedhouse et al 2001), but not in de-novo young AML patients. The majority of patients showed multifocal changes. However, not all studies have found the increased microsatellite instability in AML patients (Rimsza et al 2000). MMR defects correlated with the presence of abnormalities of chromosome 5 and 7 (Ben-Yehuda, Krichevsky et al. 1996, Das-Gupta, Seedhouse et al. 2001). A high incidence of p53 mutations was also observed in this group of patients (Ben-Yehuda, Krichevsky at al. 1996). Improper p53 function may additionally enhance genom instability.

8. Conclusions

DNA repair defects are key events in multistep evolution of the neoplastic clone. The inherited improper function of DNA repair mechanisms may lead to accelerated genom instability, including tumor suppressor genes, resulting in neoplastic transformation. Increasing understanding of mechanisms leading to development and progression of myeloid malignancies may have future implications in the modern treatment. Leukemia is frequently specified by loss of a certain pathway, which may be the target of personalized treatment. So far the modification of DNA repair in AML and MDS is still at preclinical phase. Several potential problems with strategies influencing DNA repair arise. Most

important is the tendency for secondary malignancies because of genome instability and a high risk of rapidly increasing refractoriness.

DNA repair mechanism	Detected defect	Correlation with AML	Authors
Homologous Repair	Polymorphism in genes: RAD51, XRCC3,	Increase risk of AML development	Seedhouse, Foulkner 2004
Non Homologous End Joining	Ineffective processes in patients with FLT3 mutations	Correlates with refractoriness of the disease	Seedhouse, Hunter et al. 2006
Base Excision Repair	Polymorphism in gene XRCC1 (Arg399Gln)	Protects from AML development as compared with the wild type of allele	Seedhouse, Bainton 2002
Nucleotide Excision Repair	Polymorphism in gene XPD (Lys751Gln)	Adverse effect on overall survival and disease free survival in glutamine variant	Allan, Smith 2004
Mismatch Repair	Microsatellite instability	Increases risk of secondary leukemia; correlates with high risk cytogenetics	Das-Gupta, Seedhouse et al. 2001

Table 1. DNA repair defects in AML

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10. References

Allan, J.M.; Smith, A.G. et al. 2004. Genetic variation in XPD predicts treatment outcome and risk of acute myeloid leukemia following chemotherapy. Blood 104, 3872-3877.

Ben-Yehuda, D.; Krichevsky, S. et al. 1996. Microsatellite instability and p53 mutations in therapy related leukemia suggest mutator phenotype. Blood 88, 4296-4303.

Casorelli, I.; Offman, J. et al. 2003. Drug treatment in the development of mismatch repair defective acute leukeima and myelodysplastic syndrome. DNA repair. 2, 547-559.

D'Andrea, A.D. 2010. Targeting DNA pathways in AML. Best Pract Res Clin Haematol. 23, 469-473.

Das-Gupta, E.P., Seedhouse C. et al. 2001. Microsatellite instability occurs in defined subsets of patients with acute myeloblastic leukaemia. Br J Haematol 14, 307-312.

Estey, E. 2007. Acute myeloid leukemia and myelodysplastic syndromes in older patients. J. Clin. Oncol. 25, 908-1915.

Gaymes T.J.; Mufti, G.J. et al. 2002. Myeloid leukemias have increased activity of the nonhomologous end-joining pathway and concomitant DNA misrepair that is dependent on the Ku70/86 heterodimer. Cancer Res 62, 2791-2797.

Gaymes, T.J.; Shall, S. et al. 2009. Inhibitors of poly ADP-ribose polymerase (PARP) induce apoptosis of Myeloid leukemic cells: potential for therapy of myeloid leukemia and myelodysplastic syndromes. Hematologica 94, 638-646.

Guillem, V.; Tormo, M. 2008. Influence of DNA damage and repair upon the risk of treatment related leukemia. Leuk Lymphoma 49, 204-217.

Pallis, A.G.; Karamouzis, M.V. 2010. DNA repair pathways and their implication in cancer treatment. Cancer Metastasis Rev 29, 677-685.

Rassool, F.V.; Gaymes, T.J. et al. 2007. Reactive oxygen species drive increased DNA damage and error-prone repair in a mouse model of myeloid leukemia disease progression. Cancer Res 67, 8762-8771.

Rimsza, L.M.; Kopecky, K.J. et al. 2000. Microsatellite instability is not a defining feature of acute Myelkoid leukemogenesis in adults: results of a retrospective study of 132 patients and review of the literature. Leukemia 14, 1044-1051.

Robak, T.; Wierzbowska, A. 2009. Current and emerging therapies for acute myeloid leukemia. Clin. Ther., 31, 2349-2370.

Sallmyr, A.; Fan J et al. 2008. Internal tandem duplication in of FLT3 (FLT3/ITD) induces increased ROS production, DNA damage and misrepair : implications for poor prognosis in AML.Blood 111, 3173-3182.

Seedhouse, C.; Faulkner, R. et al. 2004. Polymorphisms in genes involved in homologous recombination repair interact to increase the risk of developing acute myeloid leukemia. Clin Cancer Res 10, 2675-2680.

Seedhouse, C.H.; Hunter, H.M. DNA repair contributes to the drug-resistant phenotype of primary acute myeloid leukemia cells with FLT3 internal tandem duplication and is reversed by the FLT3 inhibitor PKC412. Leukemia 20, 2130-2136.

Seedhouse, C. ; Bainton R. et al. 2002. The genotype distribution of the XRCC1 gene indicates a role for base cision repair in the development of therapy-related acute myeloblastic leukemia. Blood 100, 3761-3766.

Seedhouse, C.H.; Das-Gupta, E.P.; Russell, N.H. 2003. Methylation of hMLH1 promoter and its association with microsatellite instability in acute myeloid leukemia. Leukemia 17, 83-88.

Shaheen, M, Allen, C. et al. 2011. Synthetic lethality : exploiting the addiction of cancer to DNA repair. Blood prepublished 2011 march 25.

Shrivastav, M., De Haro, L.P. et al. 2008. Regulation of DNA double-strand break repair pathway choice. Cell Research 18, 134-147.

Sloand, E.M. 2008 Myelodysplastic syndromes: introduction. Semin. Hematol. 45, 1-2.

Smith, A.G., Worrillow, L.J. et al. 2007. A common genetic variant in XPD associates with risk of 5q- and 7q-deleted acute myeloid leukemia. Blood 109,1233-1236.

Strom, S.S., Estey, E.H. et al. 2010. AML outcome: role of nucleotide excision repair polymorphisms in intermediate risk patients. Leuk Lympoma 51, 598-605.

Worrillow, L.J., Alla, J.M. Deregulation of homologous recombination DNA repair in alkylating agent- treated stem cell clones: a possible role in the aetiology of chemotherapy- induced leukemia.Oncogene 25, 1709-1720.

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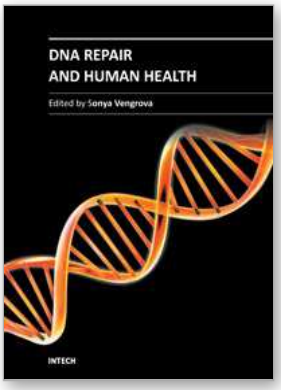

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Over the past decades, great advances have been made in understanding the cellular DNA repair pathways. At the same time, a wealth of descriptive knowledge of human diseases has been accumulated. Now, the basic research of the mechanisms of DNA repair is merging with clinical research, placing the action of the DNA repair pathways in the context of the whole organism. Such integrative approach enables understanding of the disease mechanisms and is invaluable in improving diagnostics and prevention, as well as designing better therapies. This book highlights the central role of DNA repair in human health and well-being. The reviews presented here, contain detailed descriptions of DNA repair pathways, as well as analysis of a large body of evidence addressing links between DNA damage repair and human health. They will be of interest to a broad audience, from molecular biologists working on DNA repair in any model system, to medical researchers.

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