

Summary

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Leukemia is a malignant hematological disorder characterized by proliferation of abnormal white cells that infiltrate the bone marrow, peripheral blood and other important organs. Leukemia arising from myeloid cells is known as myeloid leukemia which may either be chronic myeloid leukemia (CML) and/or acute myeloid leukemia (AML). CML is a clonal myeloproliferative disorder of hematopoietic stem cells consistently associated with the Philadelphia chromosome (Ph) resulting from reciprocal translocation of the long arms of chromosome 9 and 22 which generates a 210kDa hybrid oncoprotein (Bcr/Abl). The development of the Bcr-Abl tyrosine kinase inhibitor imatinib mesylate (Gleevec, formerly known as STI571) is the treatment of choice for chronic phase CML and its remarkable therapeutic effects suggest that the blast crisis transition will be postponed for several years in most patients with CML. AML is a malignant disease of hematopoietic system in which cells accumulate in an undifferentiated state due to mutations that prevent their normal differentiation and allow undifferentiated cells to survive and proliferate. The molecular changes that occur in AML usually lead to either abnormal cell proliferation (FLT3 and Ras mutations) or block in differentiation (AML1/ETO, PML/RAR alpha, C/EBP α mutations) or suppression of apoptosis (Bcl2 overexpression). Agents like ATRA can induce leukemic cell differentiation in other AML subtypes, although these effects may differ between patients. Therefore, screening of compounds having potential to induce apoptosis and differentiation in myeloid blasts is required that may have substantial impact in the development of better therapeutics for leukemia. The increased understanding of apoptosis and differentiation pathways has directed attention to components of these pathways as potential targets for therapeutic agents. Therefore, future studies in leukemia should focus on (i) the identification of new agents with more predictable effects on differentiation and apoptosis; (ii) the use of clinical and laboratory parameters to define new subsets of leukemic patients in which differentiation/apoptosis induction has a predictable and beneficial effect, and (iii) further characterization of how blast cells sensitivity to drug-induced apoptosis is modulated by differentiation induction. This prompted us to screen for natural/synthetic compounds for their antileukemic property. From this screening we found ormeloxifene as a potential antileukemic agent.

Ormeloxifene is a non-steroidal selective estrogen receptor modulator (SERM) and has been shown to possess potential anticancer activities in breast and uterine cancer. In the present study, we have assessed the effects of ormeloxifene in myeloid leukemia cells. We show that ormeloxifene induces apoptosis in a dose dependent manner in a variety of myeloid leukemia cells, more strikingly in K562. Our data demonstrates that Ormeloxifene induced apoptosis in K562 cells involves activation of Extracellular Signal-Regulated Kinases (ERK) and subsequent cytochrome-c release leading to mitochondria mediated caspase-3 activation. Ormeloxifene induced apoptosis via ERK activation was drastically inhibited by prior treatment of K562 cells with ERK inhibitor PD98059. Ormeloxifene also inhibits proliferation of K562 cells by blocking them in G0-G1 phase by inhibiting the expression of c-myc and enhancing the expression of cell cycle inhibitor protein p21. K562 is a chronic myeloid leukemia cell line having Bcr-Abl fusion oncoprotein which makes it highly proliferative so we sought to investigate the effect of ormeloxifene on this fusion protein. We further show that ormeloxifene inhibits the phosphorylation as well as protein expression of Bcr-Abl leading to inactivation of survival pathway. Ormeloxifene induced apoptosis is translatable to mononuclear cells isolated from CML patients. Thus, ormeloxifene induces apoptosis in K562 cells via phosphorylation of ERK and mitochondria mediated caspase activation and arrest them in G0-G1 phase of cell cycle by reciprocal regulation of p21 and c-myc. Because chronic myeloid leukemia is characterized by low apoptosis and high proliferation/survival rate, our data suggest that inclusion of ormeloxifene in the therapy of CML can be of potential utility.

Lineage differentiation in blood cells is a highly regulated process. This regulation includes multiple factors and pathways. Various transcription factors are involved in the commitment of lineage differentiation at the different stages of blood cell development. C/EBP α is one such key transcription factor required for differentiation of various cell types including myeloid cells. In addition, it is also a tumor suppressor protein involved in cell cycle regulation and apoptosis. Being a crucial factor in the neutrophil differentiation it is highly vulnerable to mutational defects that often observed in myeloid leukemia. Thus, loss of C/EBP α expression and function in myeloid cells contributes to block in differentiation leading to leukemia. Understanding the regulation of C/EBP α in myeloid cell differentiation can provide better insights in to the pathophysiology of leukemia. Post translational modifications of protein regulate its functional activity; phosphorylation, sumoylation and ubiquitination are some of the

post-translational modifications which are reported to modulate the activity of C/EBP α protein at various stages. Ubiquitination is a post translational modification where E3 ubiquitin ligases attach an activated ubiquitin to a target protein leading to its subsequent proteasome degradation. C/EBP α is reported to be ubiquitinated and subsequently degraded, however, so far Fbxw7 is the only reported E3 ligase for C/EBP α . In the present study, for the first time we demonstrate that E6AP, an E3 ubiquitin ligase also serves as an E3 ligase for C/EBP α . E6AP is a known E3 ligase involved in the ubiquitination of various cellular proteins like p53, p21 and PML-RAR α . We show that E6AP degrades C/EBP α in a dose dependent manner by ubiquitin mediated proteasome degradation; however, catalytically inactive E6AP-C843A rather stabilizes it. E6AP, an E3 ubiquitin ligase degrades C/EBP α and thereby modulates its transcriptional activity. E6AP interacts with C/EBP α and co-localizes together in the nucleus. We further show that E6AP mediated degradation of C/EBP α inhibits its transactivation potential thereby modulating its functional activity. This clearly indicates that E6AP has a direct role in the C/EBP α proteasomal degradation. E6AP mediated negative effects on the functional activity of C/EBP α are again exemplified in K562-p42C/EBP α -ER cells which stably expresses estradiol receptor ligand binding domain linked C/EBP α protein. The rate of degradation of C/EBP α in K562-p42C/EBP α -ER cells upon β -estradiol induction is decreased in the presence of E6AP-C843A. In addition, knock down of E6AP in K562-p42C/EBP α -ER cells enhances the granulocytic differentiation of these cells upon β -estradiol induction which is evident from FACS analysis for cell surface differentiation marker cd11b. Taken together, these studies substantiate the role of E6AP in the degradation and functional activity of C/EBP α . This makes E6AP an interesting protein in the study of regulation of myeloid cell development and leukemogenesis. Also, due to their target specificity these E3 ligases represent an attractive target for the cancer therapy. Therapeutic strategies that inhibit the E3 ligases activity in the degradation of tumor suppressor protein can provide a new scenario in the cancer treatment.