Development and nanotoxicological analysis of polymer/protein nanopolypharmaceuticals against Acute Myeloid Leukemia

ABSTRACT

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Persistence of a resilient, residual population of leukemic stem/progenitor cells (LSPC) is clinically implicated in the inability of current conventional chemotherapy to eradicate AML, causing relapse and therapeutic failure. In addition to residual LSPC compartment, recent line of evidences has highlighted the involvement of aberrant kinome and epigenome in leukemogenesis, which largely remains unaddressed by conventional cytotoxic chemotherapy. This thesis focuses on the development of polymer/protein nanopolypharmaceuticals targeted against deregulated signal transduction, histone deacetylation and LSPC in AML. We have investigated the prospective of a kinome targeted nanotherapy approach in clinical management of haematological malignancies, taking Acute Myeloid Leukemia (AML) as a model. Initially, to address the derailed AML kinome, we have developed a polymer-protein core-shell nanomedicine loaded with two different drugs that can inhibit critically aberrant pro-survival kinases in mTOR, MAPK and STAT pathways, in primitive (CD34+/CD38−/CD33+) AML cells. The nanomedicine consisted of poly-lactide-co-glycolide core (~250 nm) loaded with mTOR inhibitor, everolimus (Afinitor®, Novartis) and albumin shell (~25 nm thick) loaded with MAPK/STAT5 inhibitor, sorafenib (Nexavar®, Bayer & Onyx) and the whole construct was surface conjugated with monoclonal antibody against CD33 receptor overexpressed in AML. Transmission electron micrographs confirmed the formation of core-shell nanostructure (~290 nm) with distinct interphase. Flow cytometry and confocal studies showed enhanced intracellular uptake of targeted nanomedicine. Simultaneous
inhibition of critical kinases causing synergistic lethality against leukemic cells, without affecting healthy blood cells, were demonstrated using immunoblotting, cytotoxicity and apoptosis assays. This cell receptor plus multi-kinase targeted core-shell nanomedicine was found better specific and tolerable compared to clinically achieved concentrations of combination of cytarabine and daunorubicin. Subsequently, to attend the aberrant histone deacetylation in AML, we have developed a novel, protein-vorinostat nanomedicine exhibiting selective and superior anti-leukemic activity against heterogeneous population of AML patient samples (n=9), including refractory and relapsed cases, and three representative cell lines expressing CD34+/CD38- stem cell phenotype (KG-1a), promyelocytic phenotype (HL-60) and FLT3-ITD mutation (MV4-11). Nano-vorinostat having ~ 100 nm size exhibited enhanced cellular uptake rendering significantly lower IC50 in AML cell lines and patient samples, and induced enhanced HDAC inhibition, oxidative injury, cell cycle arrest and apoptosis compared to free vorinostat. Most importantly, nanomedicine showed exceptional single-agent activity against the clonogenic proliferative capability of bone marrow derived leukemic progenitors, while remaining non-toxic to healthy bone marrow cells, indicating strong possibility of nano-vorinostat to strike the resilient LSPC fraction. Consequently, well designed flow cytometric experiments of nano-vorinostat in CD34+ CD38- CD123+ cells isolated from refractory AML patients revealed complete ablation of LSPC along with blasts, without exhibiting any myelosuppressive or hematotoxic potential. Besides, nano-vorinostat also exerted excellent synergistic lethality to primary, refractory AML cells, in combination with DNA methyltransferase inhibitor, decitabine, which is approved for elderly AML patients. Considering extermination of both primitive leukemic cell and blast compartments, without causing any significant toxicity to healthy bone marrow cells, nano-vorinostat shows promise for clinical translation in the setting of a more patient compliant and effective epigenetic targeted therapeutic approach against AML.