Title: *Elucidation of the role of tumor-residing immune-suppressor cells in generation of multi-drug resistance in lymphoma with immunomodulation by neem leaf glycoprotein*

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Abstract

Non-Hodgkin-lymphoma (NHL), the most prevalent hematologic malignancies in the world, has majorly originates from B cells. For the past decade, treatment of NHL patients with R-CHOP has witnessed a significant achievement, yielding complete remission (CR) rate of 40-50%. However, due to its limited and differential response evolving from heterogeneity of NHL, a substantial population of patients undergo relapse after CR or initial treatment, resulting in poor clinical consequences. Patient's response to chemotherapy varies widely from static disease to cancer recurrence and later is primarily associated with the development of multi-drug resistance (MDR), ensuring unmet need to understand the underlying mechanisms for crafting better therapeutic strategies for relapsed disease. The immunosuppressive cells within the tumor microenvironment (TME), including myeloid derived suppressor cells (MDSCs) have become a crucial target for improving the therapeutic efficacy for various forms of cancer. However, a better understanding of their involvement is needed for distinctive response of NHL patients after receiving chemotherapy to design more effective front-line treatment algorithms based on reliable predictive biomarkers. With the purpose of identifying key constituent for relapse free patients survival, we have investigated the association of immune cells in context of cancer recurrence and therapy failure in NHL patients [pre-chemotherapy (n=10) and post-chemotherapy (n=51)]. Immunophenotypic assessment depicts the strong positive correlation between immune suppressor cells, particularly with MDSCs and MDR in non-responder cohort of NHL patients. We have accelerated our investigation and found that the patients with NHL recurrence have increased MDSC expansion and this elevated MDSC is of monocytic (M-MDSC) in nature, not granulocytic (G-MDSCs). To validate the involvement of M-MDSCs in NHL-associated MDR development, we established doxorubicin-resistant cancer cells in both *in-vitro, in-vivo* condition and executed the role of M-MDSCs in fostering drug resistance phenomenon in cancer cells. Moreover, *in-vitro* supplementation of MDSCs in murine and human lymphoma culture augments early expression of MDR phenotypes compared to culture without MDSCs, correlated well with *in-vitro* drug efflux, rhodamine accumulation, and tumor progression. We found that MDSC secreted cytokines IL-6, IL-10, IL-1β are the key regulator in development of drug resistance, thus leading to therapy failure and cancer recurrence. Moreover, we have identified that MDSC augments early drug resistance and therapy failure through IL-6/STAT1-IL-10/STAT3-IL-1β/NF-κB axis. Cumulatively, we have observed that screening of NHL recurrence patients with high titre of M-MDSCs might be considered as a new potential biomarker and treatment modality in overcoming chemo-resistance in NHL patients. Cancer recurrence and multi-drug resistance both are the crucial hurdles in the conventional treatment approach of NHL, so, we looked for an alternate approach that aims to prevent or delay MDR prior or early to its development. In our study, we have explored the role of non-toxic immunomodulator NLGP in MDSC mediated development of MDR. Our study has reported that on administering NLGP in *in-vivo* and *in-vitro* drug resistance passages, a significant downregulation was observed in the expression of MDR markers in NLGP-treated group in comparison to non-NLGP cohorts with substantial reduction in MDSC proportion. Aiming to delineate the role of NLGP and possible mechanism, we have found that NLGP have a prominent effect on both cancer cells and MDSCs in order to minimize drug resistance. NLGP alleviates drug resistance phenomena through Dectin-1 mediated NF-κB signalling, sustaining type 1 immunity.

Signature of candidate: Sukanya Dhar 06/06/2024

Anamika Bose 3/6/2024

Signature with Seal of the Supervisor

डॉ० अनामिका बोस / Dr. Anamika Bose
सहायक प्रोफेसर / Assistant Professor
फार्मास्यूटिकल टैक्नोलॉजी (बायोटैक्नोलॉजी)
Pharmaceutical Technology (Biotechnology)
राष्ट्रीय औषधीय शिक्षा एवं अनुसंधान संस्थान
National Institute of Pharmaceutical Education and Research
सैक्टर-67, एस. ए. एस. नगर-160 062, पंजाब, भारत
Sector-67, S.A.S. Nagar-160 062, Punjab, INDIA

Rathindranath Baral

Signature with Seal of the Supervisor

3/06/2024

Dr. Rathindranath Baral
Former Head
Immunoregulation & Immunodiagnostics
Chittaranjan National Cancer Institute
Kolkata; India