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Statins in Combination with Ibrutinib bypasses Resistance to Ibrutinib in Mantle Cell Lymphoma

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Abstract

Objective: In this study, we report that a novel combination therapy of different statins with Ibrutinib can overcome such resistance.

Methods: For this, we generated a cell line model, exhibiting resistance to Ibrutinib, by repeated exposure of mantle cell lymphoma cell line to Ibrutinib. Apoptosis was assessed by the extent of phosphatidylserine externalisation.

Results: Our results indicated that resistance to Ibrutinib could be overcome by targeting a key enzyme, 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase in the cholesterogenesis pathway.

Conclusion: Reusing different statins in combination with Ibrutinib could improve therapy and enhance sensitivity to Ibrutinib mediated apoptosis.

Keywords: Statins, Ibrutinib, chemoresistance, HMG-CoA reductase, cholecterogensis pathway

Introduction

Mantle cell lymphoma (MCL) is an aggressive form of malignant B-lymphocytes of non-Hodgkin's lymphoma, which arise from the outer edge of a lymph node follicle, also known as the mantle zone of lymph node. The majority of mantle cell lymphoma cases are considered as incurable with multiple incidences of relapse. Several treatment strategies have been developed over the past few years with significant improvement in patient outcome.[1–3]

Signalling of B cell receptor (BCR) is crucial for the maturation and development of B cells. Bruton's Tyrosine Kinase (BTK) is a key enzyme in B cell receptor cascade.[4–6] Many studies have identified an important role of BTK in the activation of BCR signalling pathway,[7–10] which in turn activates MAP kinase and NF-κB pathways, thereby enhancing B cell activity, survival rate, proliferation and migration.[3] Therefore, BTK is considered an important therapeutic target for MCL and other B cell malignancies.[11]

In the last few decades, various molecules have been used in clinical practice for relapsed/refractory (R/R) of MCL and other types of B cell malignancies.[10] Among these pharmacological molecules, Ibrutinib, commercially available under the name Imbruvica, is a BTK inhibitor and first line treatment for MCL.[12] However, chemoresistance to anticancer agents, including Ibrutinib poses a critical challenge for the successful implementation of this therapy. Tackling this challenge becomes vital as the resistance affects patient outcome.[13]

In recent years, several studies indicated that modulation of some cancer metabolic pathways improved cancer therapy.[14,15] Statins form an important classification of these metabolic inhibitors, and exhibit pleiotropic abilities, including anti-angiogenic, antioxidant, anti-inflammatory and anti-cancer activities.[16] Therefore, our study was aimed at identifying whether a combination of statins with Ibrutinib could overcome resistance to Ibrutinib-mediated apoptosis in MCL.

Methods

Reagents

Ibrutinib, Simvastatin and Atorvastatin were from Selleck (Sylvanfield Drive, Houston, USA). Pitavastatin was from Tocris (Abingdon, UK). Filipin III stain solution was from SigmaAldrich (Gillingham, UK).

Generation of Chemoresistance

Parental cells of MAVER-1 cells (labelled as I in Figure 1A) were exposed to Ibrutinib (10 μM) for 48h, and the extent of apoptosis was assessed using flow cytometry. This was followed by two weeks (2W) of recovery period, during which the cells were cultured in drug-free media (RPMI media + 10% FBS) at 37°C and 5% CO2. The resulting cells were labelled as II, and this process was repeated three more times more, to generate cells labelled as III, IV and V. The extent of apoptosis was checked after each exposure to assess the extent of resistance. Thus, group V cells exhibited the most resistance to Ibrutinib.

Flow Cytometry

Apoptosis was assessed by phosphatidylserine (PS) externalisation. Following 48 hours exposure to Ibrutinib and 72 hours in case of statins, cells were gathered and completed using an Annexin V buffer and propidium iodide (PI) stain. Then, the extent of apoptosis was measured by flow cytometry (Florescence-Activated Cell Sorting (FACS)).

Filipin Staining

Suspension MAVER-1 cells (0.2×10^6) were collected and resuspended with 50ml of Phosphate-buffered saline (PBS). Then, by using polysine™ adhesion slide (Menzel-Glaser, UK) the mixture was placed and left for 5 minutes. This was followed by a fixation step using paraformaldehyde (4%) from Thermo Fisher Scientific (Waltham, MA, USA) for 5 minutes and Triton X-100 (0.5%) from SigmaAldrich (Gillingham, UK) for 10 minutes. Subsequently, the slides were incubated with filipin for 2 hours, and washed three times gently with PBS, before imaging using fluorescence microscopy with a UV filter set (340–380 nm excitation, 40 nm dichroic, 430-nm long pass filter).

Statistical Analysis

Multiple comparisons one-way F test or analysis of variance (ANOVA) and the least significant difference (Fisher test)

($P \leq 0.01$) were conducted to compare sensitive (I) and resistant (V) cells. Statistics was performed using GraphPad Prism 6 software for windows (La Jolla, CA, USA).

Results

Mantle Cell Lymphoma Cells Acquired Rapid Resistance to Lbrutinib

In order to mimic the resistance observed in clinic, we developed a chemoresistance model, in which the initial MAVER-1 cells (labelled as I) were repeatedly exposed to Ibrutinib 10mM for 48h, followed by recovery periods of two weeks (2W), until increasing resistance was reached in the different groups of cells, labelled II-V (Figure 1 A and B). Taken together, these results suggested that resistance to Ibrutinib was developed successfully.

Targeting HMG-CoA Reductase Overcomes Resistance to Ibrutinib Mediated Apoptosis in Mantle cell lymphoma Cells

In order to identify a way to restore sensitivity to Ibrutinib-mediated apoptosis, we investigated whether MAVER-1 sensitive (labelled as I) and resistant (labelled as V) cells exhibited differences in cholesterol synthesis. Staining of cells with filipin indicated that cholesterol synthesis was enhanced in the resistant cells (V) compared to the sensitive cells (I) (Figure 2A). Therefore, we investigated whether targeting cholesterogenesis by three mostly used statins, namely Atorvastatin, Pitavastatin and Simvastatin could overcome resistance to Ibrutinib-mediated apoptosis in mantle cell lymphoma. Our results revealed that targeting HMG-CoA reductase by these three different statins in combination with Ibrutinib enhanced sensitivity for sensitive cells (I) and overcame resistance to Ibrutinib-mediated apoptosis in resistant cells (V) (Figure 2B). Taken together, these results suggested that resistance to Ibrutinib-mediated apoptosis could be because of the enhanced cholesterol synthesis in the resistant cells. Therefore, using statins in combination with Ibrutinib enhanced sensitivity and overcame resistance to Ibrutinib-mediated apoptosis.

Discussion

Development of resistance to cancer therapy is currently one of the main obstacle that challenges the successful anticancer therapy and is often responsible for regression and incurable cancer.[1,2,13,17] In the current study, the resistance to Ibrutinib in mantle cell lymphoma was rapidly developed (Figure 1A and B) to mimic chemoresistance in patients.

Chemotherapy often creates a stressful environment for cancer cells, which in turn respond to such unfavourable conditions by activation of several pathways that enable the cells to escape therapy.[18,19] One example of this is the rewiring of cancer metabolism. This is achieved by increasing cholesterol and lipid synthesis, in addition to activation of several other metabolic pathways.[15,18,19] For instance, in case of B cell malignancies, resistance to Ibrutinib could be achieved through the elevation of fatty acids synthesis and oxidation.[20,21] Similarly, resistance to anti-cancer agents in mantle cell lymphoma has been reported to occur via the activation of glutamine metabolism.[22] Furthermore, targeting intermediary metabolism enhances sensitivity and overcome chemoresistance.[23]

The best inhibitors of the HMG-CoA reductase are statins, such as Atorvastatin, Ptivastatin and Simvastatin, which decrease cholesterol levels in serum.[24,25] Several studies indicated that statins have the power to control growth of tumour both *in vitro* and *in vivo* by blocking the progression of cell cycle.[26–30] Furthermore, different clinical trials have been

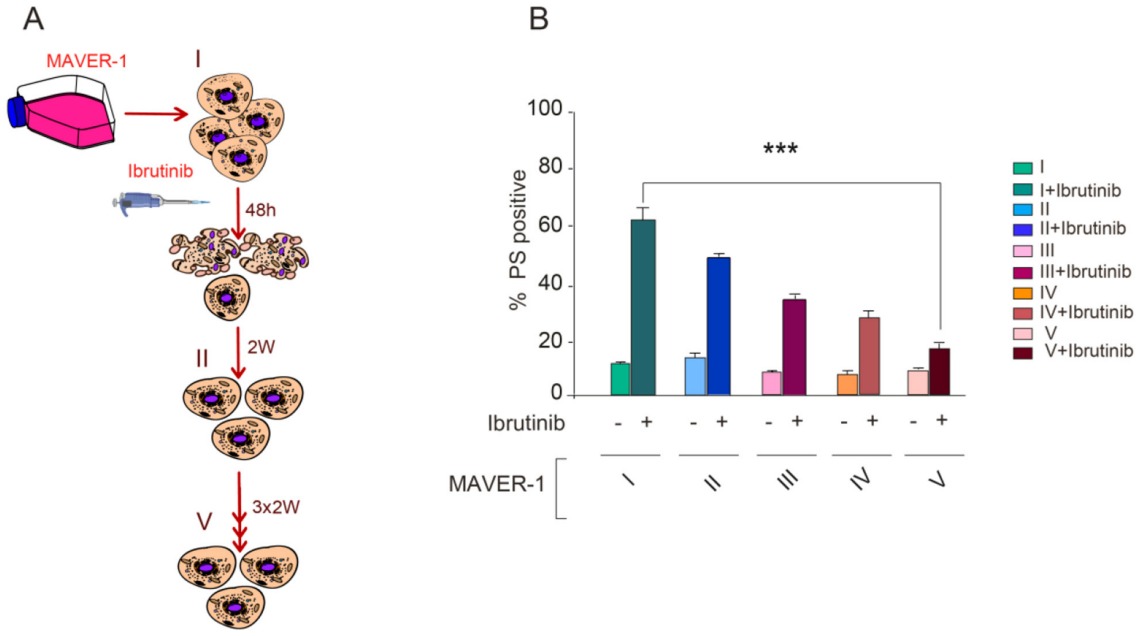

Fig.1 **Mantle cell lymphoma rapidly developed resistance to Ibrutinib. (A) Scheme for developing resistance to Ibrutinib in mantle cell lymphoma cell lines (MAVER-1), as explained in the Methods section. Sensitive (I) and resistant (V) cells of MAVER-1 cell line were exposed for 48 h to Ibrutinib (10 mM), and apoptosis was detected. (B) Gradual increase of resistance was observed in each group of cells (I, II, III, IV and V). ******P*-0.001, Error bars = mean ± standard error of mean (*n* = 3) PS: phosphatidylserine.**

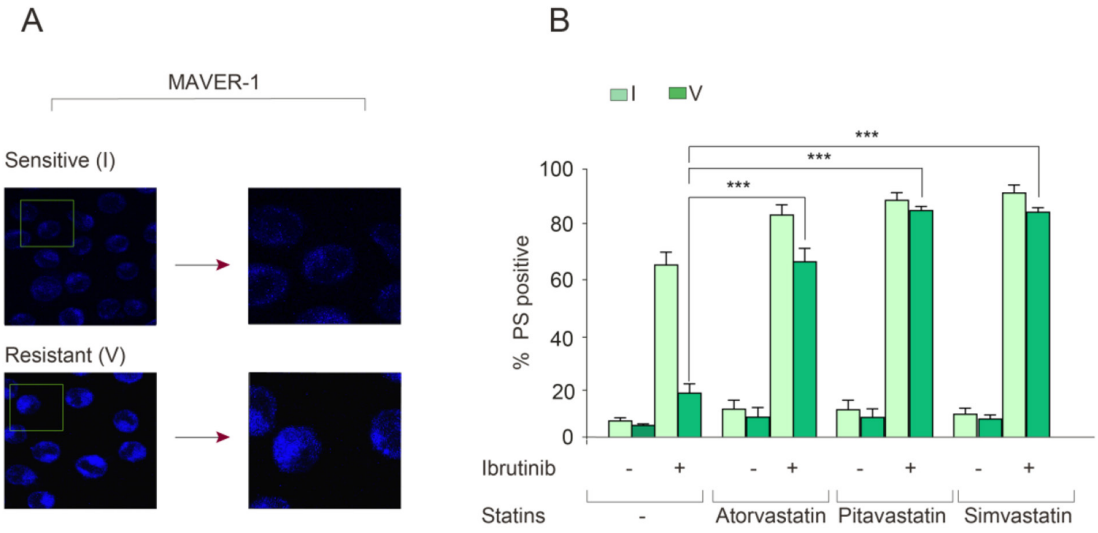

Fig. 2 **Targeting HMG-CoA reductase overcame resistance to Ibrutinib. (A) Sensitive (labelled as I) and resistant (labelled as V) MAVER-1 cells were stained with flipin (cholesterol dye) Scale bar: 10 µm. (B) Sensitive (labelled as I) and resistant (labelled as V) MAVER-1 cells were exposed to Ibrutinib (10 mM) for 48 h alone or in combination with pharmacological inhibitors of HMGR including, Atorvastatin (10 mM), Pitavastatin (1 mM) or Simvastatin (250 nM) for 72 h. ***P-0.001, Error bars = mean ± standard error of mean (n = 3). PS: phosphatidylserine.**

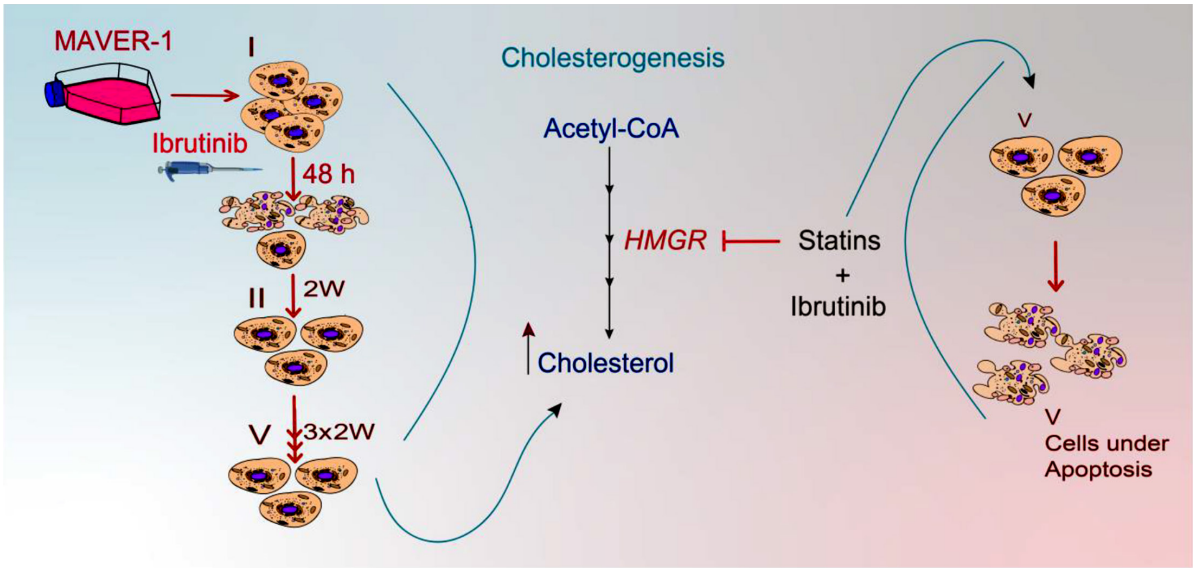

Fig. 3 **Scheme representing the development of resistance to Ibrutinib due to increased cholesterogenesis. Targeting the key enzyme HMGR by statins enhances sensitivity to Ibrutinib-mediated apoptosis.**

examined the anticancer activity of statins in many cancers, such as non-metastatic rectal cancer, head and neck cancer, advanced liver carcinoma, pediatric tumors, colon cancer and acute myeloid leukemia.[31–33] However, in the present study a group of statins was used to bypass resistance to Ibrutinib in mantle cell lymphoma cell line (Figures 2B and 3). In agreement with these results, inhibition of HMG-CoA reductase enhances sensitivity to venetoclax in different blood malignancies.[16,34]

Conclusion

Resistance to Ibrutinib mediated apoptosis was developed rapidly in mantle cell lymphoma cell line to mimic chemoresistance in patients. Increased cholesterol synthesis could be the metabolic reprogramming that MAVER-1 used to escape from therapeutic pressure presented by Ibrutinib. Therefore, re-purposing some drugs, such as statins could improve therapy and bypass resistance to Ibrutinib-mediated apoptosis.

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Competing Interests

The authors declare no competing interests.

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