Sea Cucumber Extract (TBL-12): Correlative Studies on the Anticancer Effects

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TBL-12 is currently in clinical trial at NYUCI- PI: Amitabha Mazumder, MD

Objectives

Multiple myeloma is a cancer that evolves from a state known as Monoclonal Gammopathy of Undetermined Significance (MGUS), defined by parameters of M spike and bone marrow. After evolution to myeloma, patients may be asymptomatic, that is, without any endorgan disease of hypercalcemia, renal insufficiency, and anemia or bone lesions. In asymptomatic myeloma (ASxM), there is no standard therapy. Thalidomide has been tried in patients with ASxM but with significant toxicity. Since natural agents are known to reduce toxicity while exerting chemopreventive effects, in this study we tested the chemopreventive effect of a new novel agent TBL-12 extracted from sea cucumber (Unicorn Pacific Corporation). To determine the anticancer effect of TBL-12, first we performed independent cytotoxicity assays using cell culture models**.**

Part I: Findings from our earlier studies

Cell culture

Human myeloma cells (HMCL) cells (KMS1 and ARP1) were cultured in 96 and 6 well plates in RPMI1640 medium in the presence of 10% fetal calf serum (FCS). Cells that reached 75% confluence were used to measure cytotoxic effects of TBL-12.

Treatment

Stock solution of 1mg/ml of TBL-12 extracted in DMSO (**Figure 1**) was further diluted to reach a final concentration of 25, 75, 100, 200, 250 and 500µg/ml. The cells plated in 96 sell plates treated with the above concentrations were used for MTT assays to determine the effect on cell proliferation. Similarly, cell treated in 6 well were used to perform Trypan blue (0.4%) exclusion assays to determine cell viability.

Next, the study was further extended to determine a time dependent effect on cell viability using a dose that showed an LC-50 (100µg/ml).

Fig. 1: TBL-12 and its extract in DMSO

Results

Anti-proliferative effects of TBL-12: The cellular effects induced after 48h captured under a phase contrast microscope showed remarkable decrease in the cell viability. As shown in **Figure 2A**, we observed cell death showing nuclear condensation in both KMSI and ARP1 cells indicating that the cell death could be induced through the mechanisms of apoptosis or programmed cell death in a time and dose dependent manner (however, this preliminary observation has to be confirmed with further specific assays for apoptosis).

As shown in **Figure 2B** we quantified the rate of cell death at different doses, and observed a dose dependent decrease in cell growth. Findings from MTT assay for cell proliferation is consistent the trypan blue exclusion assays for cell viability.

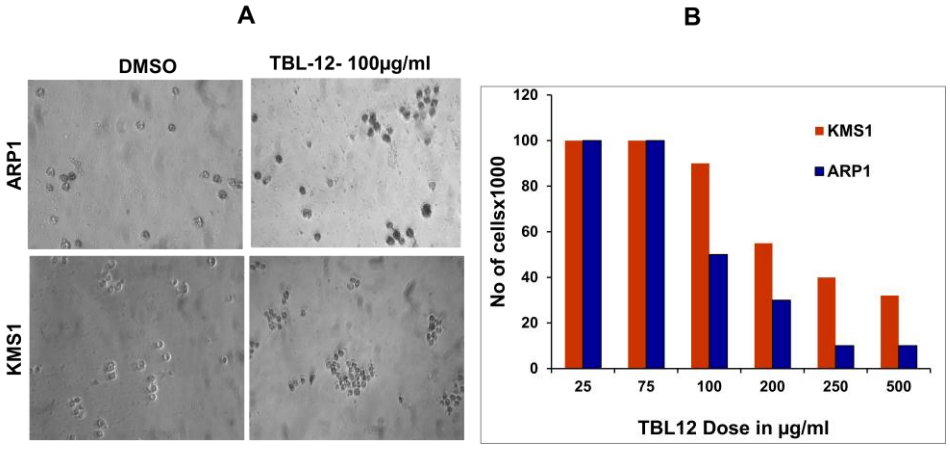

Fig. 2: Effect of TBL-12 on cell growth based on the findings from MTT assay. A: Cytotic effects of TBL-12 on myeloma cells, DMSO served as the control treated with, (B) Bar graph shows the cell growth inhibition based on MTT assay.

Part II: Current study

Based on the TBL-12 dose selected from our earlier studies we extended the analysis to determine whether similar cell growth inhibitory effects could be induced by TBL-12 in other human cancer cell types at the dose of 100ug/ml**.** To determine this we used another fast growing myeloma cell type MM1 as shown in **Figure 3** and two other human cancer cell types that represents prostate cancer (**PC-3, Figure 4),** and human cervical cancer cell type Caski, which is also positive for human papillomavirus HPV-16 (**Figure 5**). Our findings revealed that TBL-12 treatment (100ug/ml) inhibited MM1 (>50%) cell growth in a time-dependent manner.

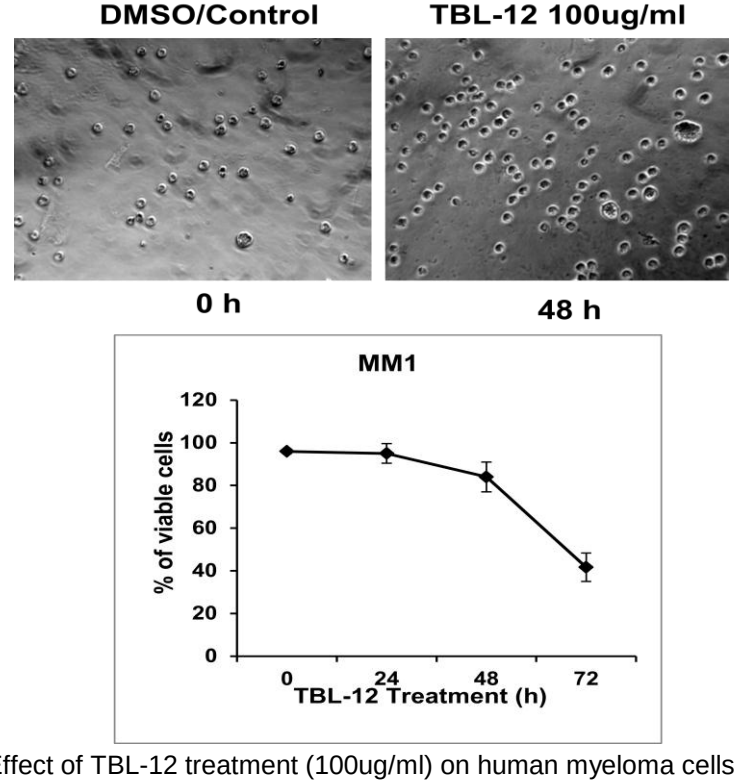

Fig. 3: Effect of TBL-12 treatment (100ug/ml) on human myeloma cells (MM1).

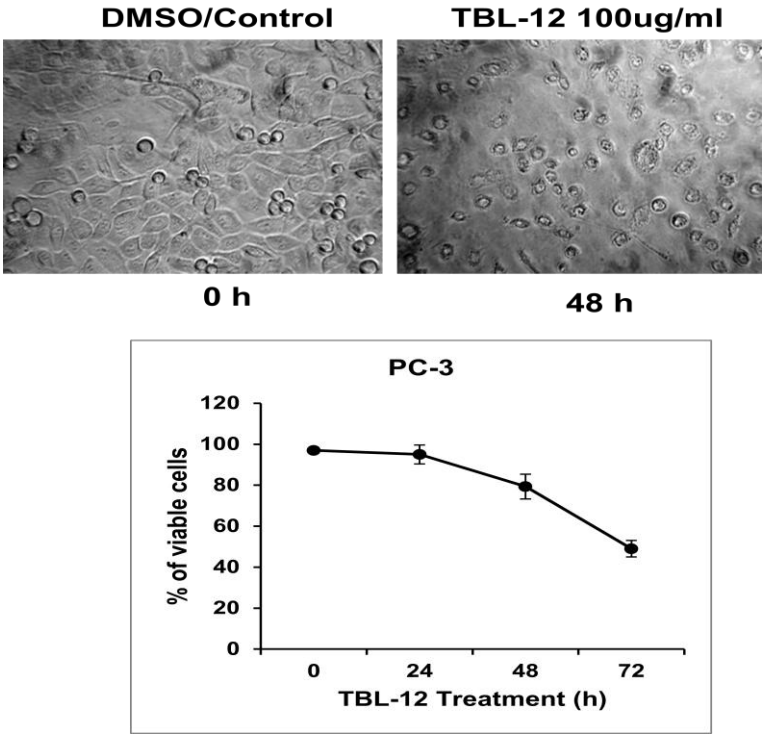

Fig. 4: Effect of TBL-12 treatment on Human Prostate Cancer Cells (PC-3)

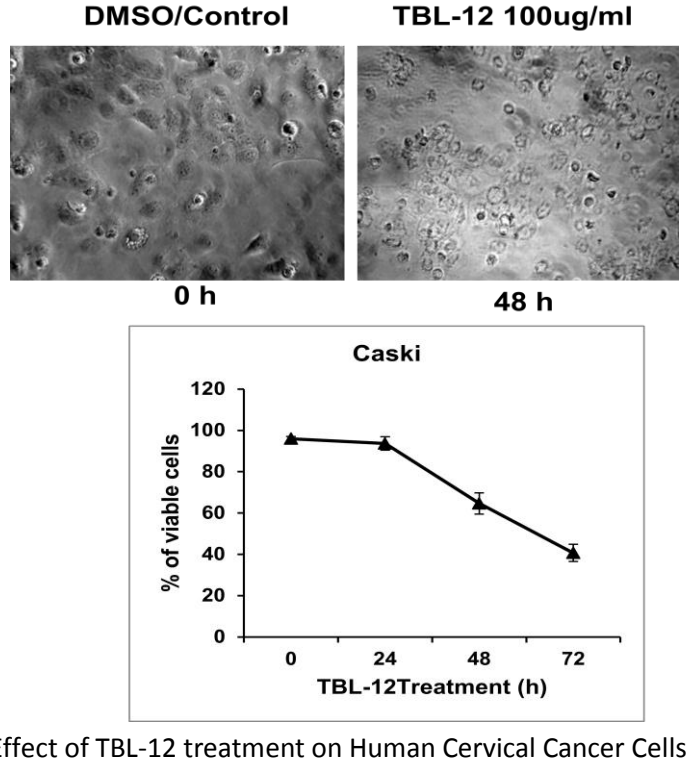

Fig. 5: Effect of TBL-12 treatment on Human Cervical Cancer Cells (CaSki)

Summary of the findings

The preliminary studies conducted in Part 1 (presented earlier) suggest that TBL-12 is effective in reducing myeloma cell growth in a dose dependent manner (Figure 2). In part II, an extended analysis of cytotoxicity assay did not reveal any necrotic effect at *in vitro* conditions using cervical (Caski) and metastatic prostate cancer (PC-3) cells, however reduced cancer cell growth determined by MTT and cell survival assays. Based on the cellular changes observed (as shown in 3, 4 and 5) it seems that TBL-12 may induce damage to the cancer cell membrane, associated with cell cycle arrest and suppression of cell division. We observed a small % of cells that goes in to a programmed cell death in contrast to necrotic cell death. Our current findings are consistent with our earlier reports in that we found an effective LD 50 dose of 100ug/ml in ARP1 cells and 200ug/ml for KMSI. In MM1 myeloma cells we observed that 100ug/ml is effective in suppressing cell growth as determined by the less number of cells in the treatment. This concentration is also found to be effective in inhibiting prostate and cervical cancer cell growth by >50% (shown line graph in figure 3, 4 and 5). Although these correlative study findings from cell based assays support the anticancer effects of TBL-12, investigating the cellular changes induced by TBL-12 could reveal several molecular targets in different human cancer.

Studies in progress

Further studies focusing on the identification of specific molecular changes induced by TBL-12 including immunological, biochemical and metabolic pathways will reveal its potential effects and health benefits. Findings from several ongoing studies will provide more insight in to the underlying anticancer mechanisms of TBL-12.