#654 Birch Bark Derivative Betulinic Acid Exerts Anti-inflammatory Activity By Targeting Mitochondrial Metabolism

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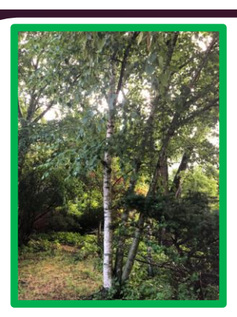
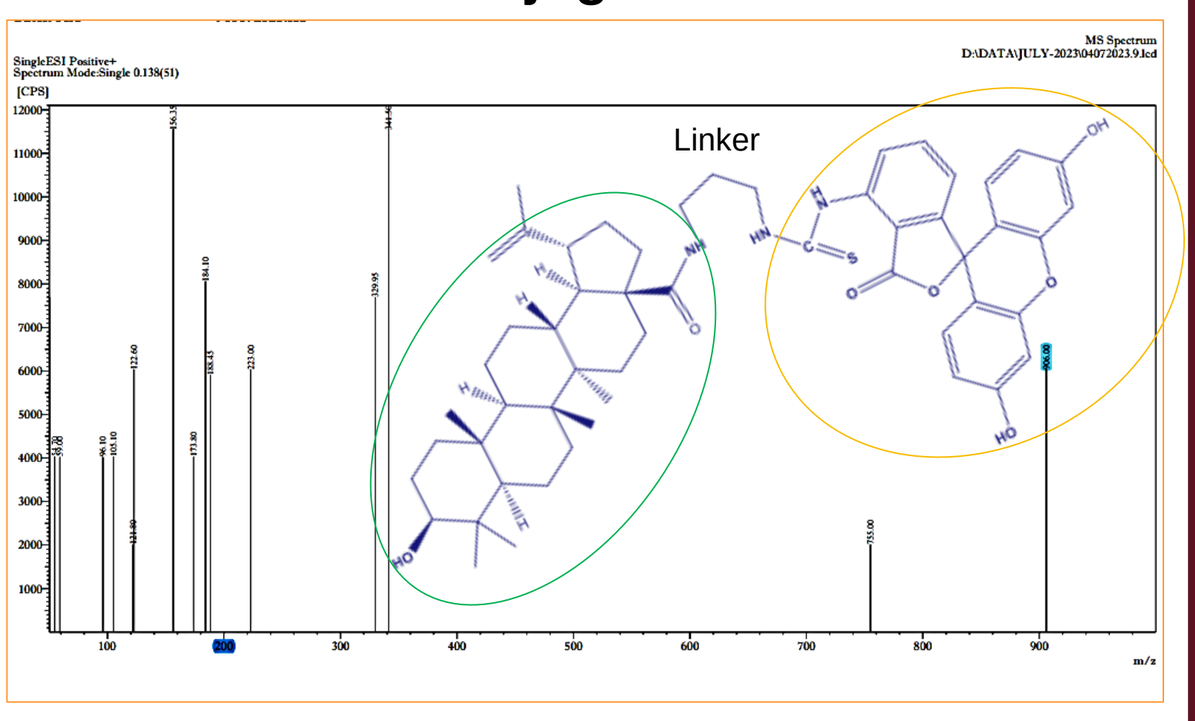


Hypothesis / aims of study

- World Health Organization (WHO) advises evidence-based use of traditional medicine such as Birch bark (*Betula alba*), backed by a long history of wound healing use by Native Indians and other cultures
- Oleogel S10 containing birch bark triterpenes earned the approval of European regulatory agency[1-2] after topical application of Oleogel S10 accelerated the chronic skin wound healing significantly more than the vehicle in a double-blind, randomized, Phase III study
- Betulinic acid is one of the triterpenes in birch bark and the clinical success of Oleogel S10 is relevant in drug development for healing lesions in Interstitial Cystitis/ Bladder Pain Syndrome (IC/BPS) patients
- Betulinic acid induces apoptosis of melanoma cells by altering mitochondrial membrane potential, which merits its selection as a topical ointment (ALS 357) in Phase I study (NCT00701987) on melanoma
- Betulinic acid is structurally similar to cholesterol- metabolized in mitochondria- and hypothesized to exert its anti-inflammatory effect by targeting mitochondria and metabolic reprogramming of immune cells
- To test the hypothesis, we covalently bonded Betulinic acid with fluorescein isothiocyanate to probe its subcellular localization with Mito tracker Red- a mitochondrial specific marker- in fast-growing immune cells and slow growing urothelium cells

Betulinic acid conjugated to Fluorescein



Study design, materials and methods

- The covalent bonding of Betulinic acid with Fluorescein isothiocyanate was confirmed by mass spectrum with dramatic increase in molecular weight of Betulinic acid
- Jurkat T immune cells from ATCC were cultured in RPMI-1640 media (Sigma Aldrich) supplemented with 10% Fetal Bovine Serum (FBS) with 1% antibiotic solution of Penicillin, Streptomycin and Amphotericin B, followed by maintenance at 37ºC in a humidified atmosphere of 5% CO2. Human urothelial cells (TRT-HU1) cells were cultured in Keratinocyte Serum Free Medium (Gibco) Cells were passaged on T25 flasks until and used within 5 passages after initial thawing.
- **Confocal Microscopy** (Olympus Fluoview FV1000): Resident urothelial cells, TRT-HU1 and Jurkat T immune cells were exposed to Betulinic acid conjugated to Fluorescein [15μM] for 4 h and then washed with phosphate buffered saline (PBS) for 15min before staining with 25nM Mito tracker Red (Invitrogen), a mitochondria marker and washed again with PBS before imaging at 60x magnification with Laser HV 454v, 2x gain and 13% offset for green and red fluorescence.
- Colocalization of green and red fluorescence was quantified by Pearson correlation coefficient and significant differences assessed by unpaired Student' t test.
- The mitochondrial action of Betulinic acid was probed further by studying the impact on gene expression of p53, Glyceraldehyde 3-phosphate dehydrogenase (GAPDH) and phosphatidylinositol 3-kinase (PI3K) relative to beta actin by real-time PCR

References

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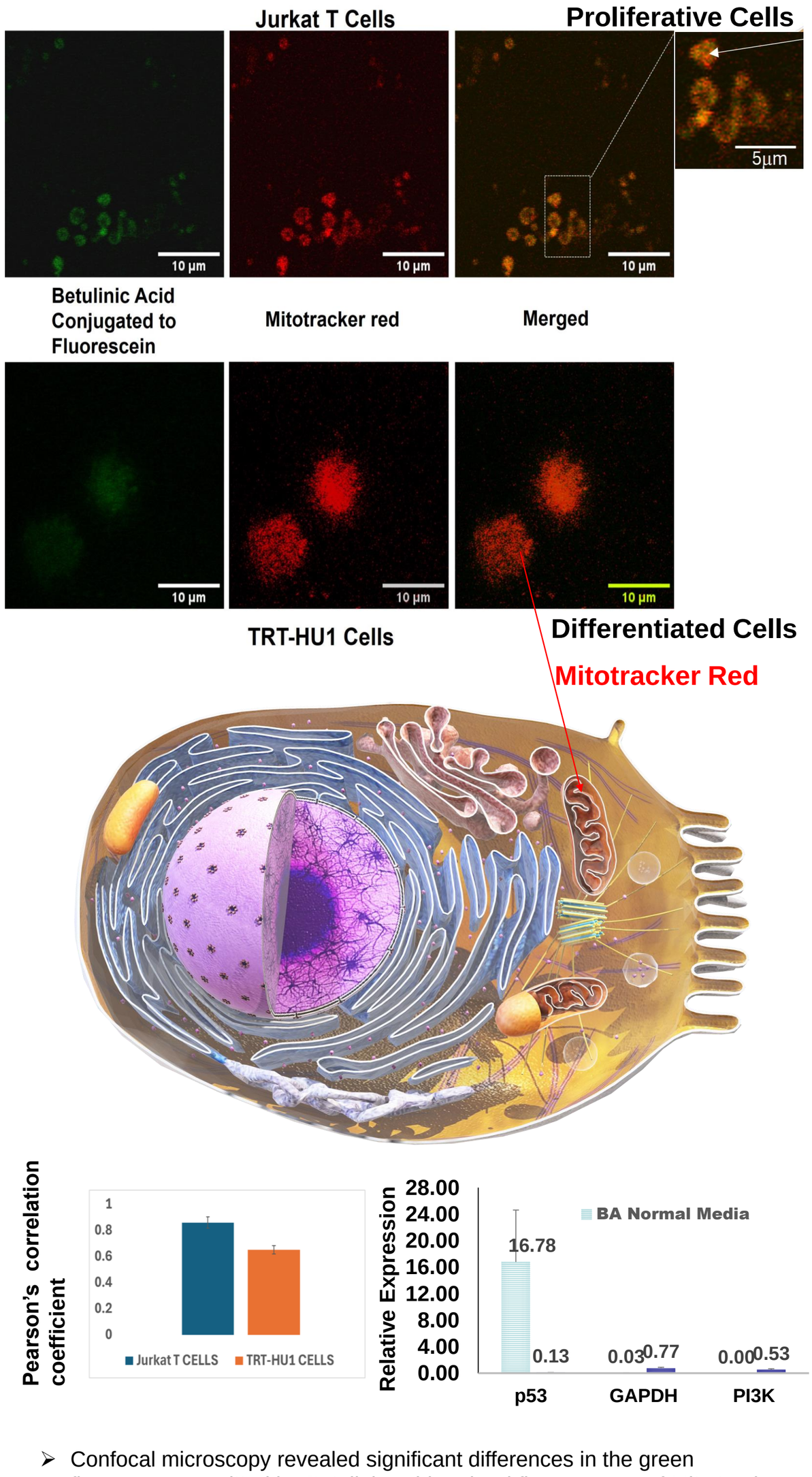
3. Ganguly, A., Daugherty, S., Healy, K., Beckel, J., Kanai, A., Tyagi, P.: Anti-inflammatory effect of Betulinic Acid Is Dependent on Metabolic Reprogramming of Immune Cells. J. Urol, 211, 2024
4. Jiang T, Zhou X, Chen Z, Xiong T, Fu J, Liu Z, Yan D, Zhou Z, Shen W. Clinical efficacy of submucosal injection of triamcinolone acetonide in the treatment of type II/III interstitial cystitis/bladder pain syndrome. BMC Urol. 2020 Mar 30;20(1):36.

Results and interpretation



- Confocal microscopy revealed significant differences in the green fluorescence emitted by Betulinic acid and red fluorescence of Mito tracker red emitted by mitochondria of fast-growing smaller immune cells (doubling time of 23.1h) and slow growing but larger urothelium cells (doubling time of 44h)
- Mitochondrial accumulation of fluorescent Betulinic acid in fast growing immune cells implies that two-fold faster cell division consumes nutrients at a higher rate than urothelium to create nutrient scarcity which triggers metabolic signals of pseudo-starvation and induction of epigenetic changes (manifesting as the downregulation of GAPDH gene and PI3K in conjunction with p53 gene upregulation) signifies rewiring of metabolic pathways (metabolic reprogramming) to fuel growth
- Betulinic acid mediated p53 upregulation is consistent with the mitochondrial membrane potential changes, production of reactive oxygen species, and the opening of mitochondrial transition pores for the release of apogenic factors, activation of caspases, and DNA fragmentation
- Preferential uptake of Betulinic acid as a metabolic fuel for driving cell proliferation in nutrient-poor environments at inflammatory foci[1] supports the role of metabolic reprogramming in the anti-inflammatory effect
- The nutrient scarcity experienced by immune cells infiltrating into inflammatory foci on bladder mucosa can be inferred from venous congestion noted on bladder biopsy specimens of IC/BPS patients, a hallmark of constrained supply of nutrients and oxygen in tissue

Conclusions

- Significantly higher co-localization of green fluorescence emitted by Betulinic acid with the red fluorescence of Mitotracker red in fast growing immune cells than in the slow-replicating urothelium cells substantiates the role of altered gene expression of p53, GAPDH and PI3K in the anti-inflammatory effect of Betulinic acid
- The wound healing associated with recurrent topical administration of birch bark derivatives in recent Phase III study(ref.1-2) could have been partly contributed by Betulinic acid's action on mitochondria of immune cells
- Findings imply that self-assembling attribute of Betulinic acid into nanoparticles can be capitalized to deliver anti-inflammatory steroids directly to the immune cells of urothelium via intravesical administration to IC/BPS instead of cystoscopic guided injection of steroids[4].
- This research complies with the WHO mandate for scientific rigor in research into traditional medicine of Birch bark