

expressed and purified in *E. coli* using a GST tag system. SDS-PAGE and western blot were conducted to detect the recombinant L-FABP T94A variant.

Results: Gel electrophoresis confirmed that the *E. coli* contained the recombinant pGEX-6p-2/L-FABP. Digestion with restriction enzymes *Bam*HI and *Xho*I showed two bands, 4900bp was the pGEX-6p-2 and 418bp was the L-FABP. After site-directed mutagenesis, plasmids were transformed into competent DH5 α cells, which were then grown on agar plates. The results indicate that the mutant plasmid was properly transformed. DNA sequencing of original and mutated rat L-FABP showed the substitution of the threonine position 94th was substituted for an alanine in the mutated DNA. SDS-PAGE and western blot confirmed proper purification of L-FABP and L-FABP T94A variant.

Conclusion: Replacement of threonine with alanine at the 94th amino acid (T94A) of rat L-FABP was properly constructed. Original and site-directed mutant rat L-FABP was successfully purified.

3. Tyrosine Kinase Inhibitors Decrease Molecular Biomarkers of Fibrosis in Activated Hepatic Stellate Cells *in vitro*

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Purpose: Hepatic fibrosis is a worldwide problem threatening human health due to the risk of complications. Hepatic injury stimulates a wound-healing response and continuous injury results in an excessive accumulation of extracellular matrix (ECM), hepatic fibrosis and ultimately cirrhosis or other serious hepatocellular diseases. The Tyrosine Kinase Inhibitors (TKIs) have shown antifibrotic effects and may represent a new generation of antifibrotic drugs. In computational analyses we demonstrated for Ibrutinib and Dabrafenib, two recently approved anticancer TKIs, had more favourable binding energies to PPAR γ than the known PPAR γ agonist, Rosiglitazone, which also has reported antifibrotic effects in the liver. The nuclear receptor, peroxisome proliferator-associated receptor-- γ , is involved in cell proliferation and ECM production. Based on the above observations, we hypothesize that Ibrutinib and Dabrafenib suppress hepatic stellate cell activation via

PPAR γ agonism.

Methods: To confirm *in silico* results, we purchased a commercially available PPAR γ competitive binding assay kit and a Cignal Reporter assay kit to assess Ibrutinib's and Dabrafenib's binding affinity and transactivation potential of PPAR γ , respectively, using rosiglitazone as positive control. The HepG2 cell line, which expresses adequate endogenous PPAR γ , was used for assessment of transactivation potential. To assess the antifibrotic effects *in vitro*, we determined the expression of fibrosis biomarkers and PPAR γ using qPCR in both quiescent and activated human hepatic stellate cells (LX-2) treated with Ibrutinib and Dabrafenib at various concentrations.

Results: The binding EC₅₀ values of Ibrutinib and Dabrafenib exceeded solubility limits and 100 μ M, respectively. Ibrutinib caused limited transactivation of PPAR γ while Dabrafenib failed to transactivate PPAR γ in HepG2 cells. Ibrutinib and Dabrafenib decreased the mRNA expression of fibrosis biomarkers in activated LX-2 cells, but not PPAR γ .

Conclusion: Based on our study, Ibrutinib and Dabrafenib are poor agonists of PPAR γ . Reductions in fibrotic biomarkers suggest possible antifibrotic effects which do not involve the PPAR γ pathway.

4. Zirconium-89 Labeled Gene Delivery Nanoparticles as Theranostic Agents for Melanoma

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Purpose: The overall approach to use targeted nanoparticles as carriers, for both therapeutic and contrast agents is that drug delivery and efficacy can be monitored simultaneously in a non-invasive manner. The goal of our research was to develop a specific Zirconium-89 (⁸⁹Zr) -labeled DNA delivery nanosystem and clarify its *in vitro* and *in vivo* behavior.

Methods: DNA containing nanoparticles were formulated using peptide-modified gemini lipid and deferoxamine modified gemini lipid. The

nanoparticles were characterized by dynamic light scattering, zeta potential analyzer and wide-angle X-ray scattering and radiolabeled with ^{89}Zr . Radiolabeling efficiency, serum stability, gene expression and cell viability were investigated in A375 human melanoma cells. Biodistribution and pharmacokinetic profile were examined in athymic CD-1 nude mice by gamma counter.

Results: The average hydrodynamic size of the nanoparticles was 114 ± 2 nm and zeta potential $+31.63 \pm 1.4$ mV. A high labeling stability of the ^{89}Zr -labeled nanoparticles was observed, and gene expression was similar to the non-labeled formulations. The nanoparticles showed longer plasma half-life ($t_{1/2} = 10.1 \pm 0.4$ h vs. $t_{1/2} = 1.3 \pm 0.1$ h) compared to the lipid alone. As expected, the ^{89}Zr -labeled nanoparticles showed higher liver accumulation ($33.87 \pm 2.20\%$ ID/g vs $20.75 \pm 5.35\%$ ID/g,) and lower kidney accumulation ($0.96 \pm 0.08\%$ ID/g vs $1.34 \pm 0.16\%$ ID/g) of the compared to radiolabeled lipid, which suggests that the nanoparticles remained intact in the blood, and these intact nanoparticles spend sufficient time in the blood stream for a specific tissue accumulation.

Conclusions: The DNA containing radiolabeled delivery system was successfully prepared. The labeled nanoparticles were stable and showed similar in vitro characteristics to the non-labeled nanoparticles. It was established that the intact nanoparticles have significantly different pharmacokinetic and accumulation profile compared to the lipid and show potential for applications in targeted cancer gene therapy.

5. Disparate Effects of Resveratrol and Analogues (Pterostilbene and Gnetol) in the Spontaneously Hypertensive Heart Failure (SHHF) Rat

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Purpose: Significant research interest to date has focused on the polyphenol, resveratrol (trans-3,5,4'-trihydroxystilbene), a stilbenoid that is purportedly linked to improved longevity and cardiovascular

health. Although resveratrol is well-tolerated in humans, it is readily metabolized and exhibits low bioavailability. Therefore, we queried whether resveratrol analogues would produce greater vasculoprotective effects in an experimental model of hypertension and heart failure.

Method: Sprague-Dawley (SD) and SHHF rats (n=8) were treated for 8 weeks by gavage with vehicle control (C) or low doses (2.5 mg/kg/d) of resveratrol (R), pterostilbene (P), and gnetol (G). Blood pressure was measured by tail-cuff plethysmography. Animals were anesthetized and third-order mesenteric resistance arteries were isolated. Vascular function, structure and mechanical properties were evaluated by pressure myography.

Results: Systolic blood pressure was increased in the SHHF rat (196 ± 3 mm Hg, vs. SD 142 ± 7 mm Hg, $p < 0.01$), and was unaffected by stilbenoid treatment. Lumen diameters were reduced in SHHF vessels (200 ± 5 μm vs. SD 318 ± 9 μm , $p < 0.01$). As media cross-sectional area was unchanged, media-to-lumen ratios increased (SHHF 17.4 ± 1.2 vs. SD 8.9 ± 0.5 , $p < 0.01$); these changes mimic the “eutrophic remodelling” that occurs in resistance arteries from patients with essential hypertension. Lumen narrowing in SHHF arteries was attenuated by resveratrol and pterostilbene (SHHF-R 237 ± 6 μm and SHHF-P 238 ± 6 μm , $p < 0.01$), but not gnetol (SHHF-G 223 ± 9 μm), whereas media-to-lumen ratio was reduced toward normal by all three stilbenoids (SHHF-R 13.8 ± 0.8 , SHHF-P 13.7 ± 0.5 , SHHF-G 13.0 ± 0.3 , $p < 0.01$).

Conclusion: The mesenteric resistance arteries of the SHHF rat exhibit eutrophic remodeling, modeling small artery disease in human essential hypertension. Resveratrol, pterostilbene, and gnetol failed to lower blood pressure; importantly, this suggests that vascular improvement was not secondary to blood pressure lowering, but rather a result of direct actions on the arterial wall. Thus, further research on stilbenoid polyphenols as an adjunct to current anti-hypertensive therapy is warranted.