Chapter 1

Introduction

1.1. Background

In 1971, professor Judah Folkman, having noted a common observation in the field that that new blood vessels would accompany the growth of malignant tumors, proposed the hypothesis that the tumor and its vasculature constitute a "highly integrated ecosystem" in which the tumor stimulates the growth of capillaries that in turn provide blood flow to fuel its expansion (Ansari et al., 2022; Han et al., 2025; Liu et al., 2023). After several studies elucidating the existence and role of growth factors and signals involved in angiogenesis, Folkman proposed again in 1995 that tumor growth was marked by an "angiogenic switch", an event in which pro-angiogenic factor secretion is exacerbated to allow the tumor to grow (Dudley & Griffioen, 2023; Liu et al., 2025). Later research established a threshold for this phenomenon to be a diameter of 1–2 mm, by which tumors without neighboring vascular networks would remain benign or at lower stages of development. By the early 2000s, anti-angiogenic therapeutics received FDA approval for cancer treatment, and tumor angiogenesis was named a hallmark of cancer development in a seminal paper by Hanahan and Weinberg (Geindreau et al., 2022; Hanahan, 2026; Liu et al., 2023).

Most FDA-approved anti-angiogenics target the Vascular Endothelial Growth Factor (VEGF) signaling pathway, named after a growth factor identified in 1979 that was found to stimulate angiogenesis and increase vessel permeability (Lee et al., 2025; Mabeta & Steenkamp, 2022; Roskoski, 2025). VEGF signaling is ultimately responsible for the cellular proliferation and migration necessary for vascular endothelial cells to perform angiogenesis, and in cancer is associated with poor prognosis and immunosuppression (Liu et al., 2023; Mahaki et al., 2025; Yang & Cao, 2022). The first anti-angiogenic treatment to receive approval, bevacizumab, is a monoclonal antibody targeting VEGF isoform A, with more monoclonal antibodies and small-molecule RTK inhibitors targeting VEGF receptors following

shortly after (Lee et al., 2025; Shaw et al., 2024). Despite preclinical success, many anti-VEGF therapies are susceptible to chemoresistance in the form of compensatory angiogenesis using fibroblast growth factor (FGF) and platelet-derived growth factor (PDGF) as alternative pathways, resulting in poor performance in clinical settings (Izadpanah et al., 2025; Liu et al., 2025; Yang et al., 2025). Therefore, there exists ample demand for improvements to anti-angiogenic therapy, such as co-administration with immunotherapy and nanoparticle formulations, to overcome concerns of chemoresistance and toxicity (Sun et al., 2023; Rashidi et al., 2024; Zhang & Brekken, 2022).

Heparin-folate nanoparticles (HF-NPs) are nanoparticles consisting of heparin, a naturally occurring glycosaminoglycan indicated as an anti-thrombotic for clotting disorders, and folic acid, a metabolite required for DNA synthesis for which receptors are frequently expressed in tumor cells (Heyden et al., 2024; Koirala et al., 2025). Wang et al. (2015) first described the self-assembly of heparin and folic acid into nanoparticles capable of bearing indocyanine green, suggesting HF-NPs may be a cost-effective method of achieving the tumor-targeting specificity of nanoparticles. However, nanoparticles have been discovered to exert anti-angiogenic effects themselves, an effect which may act as a confounding factor for evaluating the potential for HF-NPs to deliver anti-angiogenic drugs (Saeed et al., 2019). To account for this variable, this investigation is aimed at investigating the effects of empty heparin-folate nanoparticles delivered with small-molecule anti-angiogenics in the absence of encapsulation.

1.2. Objective

The objective of this investigation is to assess the interaction between the effects of empty heparin-folate nanoparticles and anti-angiogenic therapeutic sorafenib tosylate on MAPK pathway expression and angiogenesis using *in-vitro* cell culture models.

1.3. Hypothesis

The functioning hypotheses of this study are as follows:

H_0: Heparin-folate nanoparticles will not affect the anti-angiogenic activity of sorafenib tosylate, as indicated by a lack of significant change in expression of p-PI3K, p38 and Erk, as well as no change in angiogenic activity.

H_A: Heparin-folate nanoparticles will increase the anti-angiogenic activity of sorafenib tosylate, as indicated by a decrease in expression of p-PI3K, p38 and Erk, as well as reduction in angiogenic activity.