I. INTRODUCTION

1.1 Background

Pancreatic ductal adenocarcinoma (PDAC) is of extremely poor prognosis, with a five-year survival rate lower than 10% (Nakaoka et al., 2023). This has led to an amount of cancer deaths that encroaches yearly incidence of PDAC; 466,000 cases of mortality and 496,000 new cases in 2020 (Sung et al., 2021). This high mortality rate is attributed to the challenges associated with detecting PDAC in an early stage, with most patients presenting at the advanced or metastatic stage at the time of diagnosis, which typically develop therapeutic resistance (Nakaoka et al., 2023; Peng et al., 2016). Notably, PDAC is typically asymptomatic and only clinically manifests once it initiates tissue invasion or metastasis (Martinez-Useros & Garcia-Foncillas, 2016). An early diagnosis of PDAC is imperative in maximising patient survival rates, as evidenced by reports stating that the five-year overall survival (OS) rate of patients with stage 0, Ia, and Ib of disease are 85.8%, 68.7%, and 59.7%, respectively; much higher than the OS rates of patients with higher stages of disease (stage >II) (Ushio et al., 2021).Thus, it would be pivotal to identify non-invasive novel diagnostic biomarkers that would enable the identification of PDAC at an early stage (Sturm et al., 2022).

Considering this unmet clinical need, as well as the fact that the cell signalling pathways which underlie the onset and progression of PDAC have yet to be determined, it is imperative to unravel these cell signaling pathways to establish these novel biomarkers, as well as identify new therapeutic targets which can increase patient prognosis even at advanced stages of disease (M. C. Jiang et al., 2019; Principe et al., 2021; Wong et al., 2022). Furthermore, considering that >98% of the human genome comprises of non-protein coding regions which generate noncoding RNAs (ncRNAs), it may be worthwhile to divert attention to identify the possible oncogenic roles that ncRNAs may possess (Barik et al., 2021). Notably, amongst the two primary types of ncRNAs, particular emphasis should be emplaced upon long noncoding RNAs (lncRNAs) possessing a transcript size longer than 200 nucleotides (Xiao et al., 2016). This is considering that a growing number of studies have reported that the differential expression or mutated lncRNAs are implicated in the tumorigenesis of a variety of cancers (Prensner & Chinnaiyan, 2011; Yang et al., 2014). In particular, the tumorigenic properties of lncRNAs are specific to certain cell types, and encompasses modulations at transcriptional, post-transcriptional, and epigenetic levels (Kunovsky et al., 2018).

Moreover, it is interesting to note that lncRNAs are capable of acting as conditional or constitutive tumour suppressor genes or oncogenes, and possess the capacity of regulating all cancer hallmarks (Aprile et al., 2020). This regulation would also not simply be limited to cellular processes such as apoptosis, proliferation, migration, invasion, and cancer cell stemness, but also constitutes alterations of the tumour microenvironment (M. C. Jiang et al., 2019; Liu et al., 2021). However, despite rapid progress being made in identifying differentially expressed and mutated lncRNAs in cancer, progress in identifying their exact function in tumorigenesis remains to lag far behind (Aprile et al., 2020). This disparity is a consequence of lncRNAs possessing a wide array of functions, which includes (1) signals which regulate gene expression, (2) decoys that limit the availability of specific regulatory factors, (3) scaffolds which facilitates the assembly of multicomponent complexes, (4) guides that directs ribonucleoprotein complexes to specific target genes, (5) stabilisers that bind to mRNA molecules to both stabilise and promote their translation, and (6) RNA sponges which bind and sequesters miRNA

to limit their availability and downstream effects (Aprile et al., 2020; Barik et al., 2021; Fang & Fullwood, 2016; McCabe & Rasmussen, 2021).

The complexity associated with identifying the exact oncogenic role of lncRNAs is also attributed to fact that regulatory functions of lncRNAs can also depend on subcellular localisation (Barik et al., 2021). Notably, nuclear lncRNAs regulate gene expression on a transcriptional level via epigenetic and histone modifications, as well as by recruiting or decoying certain transcription factors, whereas cytoplasmic lncRNAs regulate gene expression on a translational level by directly interacting with the translational machinery itself (Aprile et al., 2020). Moreover, this complexity is further exacerbated by the fact that the expression of lncRNAs is frequently specific to distinct cell types, tissues, developmental periods, and diseases (Balas & Johnson, 2018). In addition, *In silico* analysis of lncRNA has also exhibited that their capacity in dysregulating key tumorigenic pathways is only disruptive in specific tumour contexts, with this context-specificity being as a result of the ability of lncRNAs to engage with multiple mechanisms of regulating gene expression (Segal & Dostie, 2023). Therefore, the signalling pathways which lncRNAs regulates is generally tissue-specific, and are not conserved between different cancer types. For instance, the lncRNA, terminal differentiation-induced non-coding RNA (TINCR) has been previously found to be oncogenic in breast, bladder, gastric, and oesophageal cancers, and possess tumour suppressor functions in prostate cancer, retinoblastoma, and gliomas (Ghafouri-Fard et al., 2020).

Consequently, this necessitates the need to study the functional characteristics of lncRNAs individually in different types of cancer, in spite of any preceding data which may report on its specific tumorigenic properties in another type of cancer. This applies to LncRNA X, a novel hypoxia-inducible lncRNA whose role in the onset and progression of PDAC have yet to be studied. Considering the 88% of all pancreatic cancers are hypoxic and overexpress hypoxia inducible factor-1 (HIF-1), as well as the role of HIF-1 signalling in elevating tumour invasiveness and progression, the importance of studying LncRNA X in PDAC cannot be simply understated (Ali et al., 2022; Erickson, Highsmith, et al., 2015; Li et al., 2019). By investigating the tumorigenic pathways that LncRNA X plays a role in, it would be possible to identify its potential as a novel and early diagnostic and prognostic biomarker for PDAC. Furthermore, considering that HIF-1 signalling significantly boosts cancer malignancy by activating angiogenic, invasive, metastatic, and stemness properties in cancer cells, there is an inherent potential of utilising LncRNA X as a therapeutic target for PDAC by silencing aberrantly overexpressed HIF-1 signalling pathways (Lin, 2020; Xiong et al., 2020). Considering these factors, it is undeniable that there is great potential of improving PDAC diagnosis and prognosis by studying LncRNA X in PDAC.

1.2 Objectives

The primary objective of this research project is to investigate the role of LncRNA X in the malignancy of PDAC, including its role in cancer cell migration, invasion, and stemness via transwell migration assay and high-throughput RNA sequencing technologies. This was performed to identify the potential of LncRNA X as a novel biomarker for PDAC, as well as determine the tumorigenic pathways that it plays a role in.

1.3 Hypothesis

It is hypothesised that LncRNA X is able to promote cancer cell migration and engage in HIF-1 signalling pathways in PDAC, with HIF-1-related pathways being diminished with downregulation of LncRNA X.