Review Paper:

MiR-21: Driving lung cancer progression through hallmarks and its therapeutic potential

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Abstract

Lung cancer, a leading cause of cancer-related mortality globally, poses significant challenges due to its varied subtypes and limited treatment options. miR-21, a microRNA with oncogenic properties, plays a crucial role in various cellular processes essential for cancer progression. This review delves into the role of miR-21 in lung cancer progression, highlighting its regulation of key cancer cell functions such as sustaining proliferative signaling, evading growth suppressors, resisting apoptosis, enabling limitless replication, inducing angiogenesis and promoting invasion and metastasis.

Furthermore, miR-21's impact on immune regulation underscores its potential as a therapeutic target. By targeting miR-21, researchers aim to develop innovative strategies to enhance treatment outcomes for lung cancer patients.

Keywords: miR-21, Cancer hallmarks, Cancer progression, Therapeutic target, Lung cancer.

Introduction

Cancer is a growing global crisis, with an estimated 19.3 million new cases and nearly 10 million deaths attributed to the disease in 2020. Cancer epidemiology varies globally due to environment, lifestyle, genetics and healthcare availability[51]. The advancements in cancer treatment have improved outcomes, but the complexity of cancer necessitates further exploration of novel therapeutic avenues. Cancer cells acquire specific "hallmarks" capabilities that allow them to grow and spread uncontrollably. These hallmarks include sustaining proliferative signaling, resisting cell death, evading growth suppressors, enabling angiogenesis, inhibiting apoptosis and triggering invasion and metastasis. Researchers aim to develop therapies that disrupt the fundamental processes driving cancer progression by targeting these hallmarks.

Based on estimates from the Global Cancer Repository (GLOBACON), lung cancer is the leading cancer for men while breast cancer takes the top spot for women. Also, lung cancer remains a significant concern for women, ranking fifth position in overall incidence[42]. Despite being a leading cause of death globally, lung cancer has seen significant advancements in diagnosis and treatment over recent decades. Targeted therapies offer a promising approach, but their application is currently limited to a specific group of non-small cell lung cancer (NSCLC) patients with driver mutations.

The majority of patients lacking these mutations are often present with advanced-stage disease and chemotherapy with or without radiotherapy remains the primary treatment option. Unfortunately, while targeting cancer cells, these chemotherapeutic agents also impact other rapidly dividing cells in the body, leading to undesirable side effects[32]. Therefore, current treatment strategies fall short of adequately managing side effects, necessitating the development of improved therapies.

microRNAs: Tiny messengers

microRNAs (miRNAs) are tiny non-coding RNA molecules that are highly conserved and act as master regulators of gene expression. They are about 19 to 25 nucleotides in length that pair with complementary sequences of messenger RNAs (mRNAs) and influence gene activity. miRNAs play a role in various cellular processes, like maintaining cellular equilibrium in gene expression and ensuring cells respond appropriately to signals. However, they are found in fragile DNA sites that make them prone to alterations like deletions or duplications in cancer cells[3]. Cancer-linked miRNAs can be either oncogenic (promoting cancer) or tumor-suppressive (preventing cancer). Their segregation depends on interfering with carcinogenesis-related processes like cell migration, invasion, apoptosis and proliferation[18].

Researchers discovered miR-15a and miR-16 as the first miRNAs that suppress tumor growth. They control *Bcl-2*, often overexpressed in cancers like leukemia and lymphoma[9]. In tumors, miRNA levels are abnormal compared to healthy cells. Less developed tumors tend to have lower miRNA levels than their more advanced counterparts. Despite these lower levels, miRNA patterns accurately distinguish and classify different cancer types, outperforming mRNA-based methods[3]. Two advanced strategies in the field of miRNA therapeutics are emerging for cancer treatment. The sandwich RNA inhibition approach combines siRNAs (small interfering RNAs) and miR-mimics to target a specific molecular defect associated with cancer precisely.

On the other hand, the multiplex RNAi inhibition strategy targets multiple molecular defects in cancer pathways. For instance, in Kristen Rat Sarcoma (*KRAS*)-mutant colorectal cancer, sensor siRNAs have successfully targeted the Rapidly Accelerated Fibrosarcoma (*RAF*) node. miRNA

therapy utilizes various tools involving anti-miRNA molecules to restore the function of tumor-suppressing genes or miRNA-mimics to suppress oncogenic genes. MiRNA inhibitors (e.g. miRNA antisense therapy) and sponges work by altering dysregulated gene expression in cancer, thereby blocking the processing of endogenous miRNAs and preventing their action. They include RNA, DNA and DNA analogs[46].

Based on observations, different miRNA inhibitors (antagomiRs) have shown enhanced anti-tumor effects. Additionally, some inhibitors can sensitize drug-resistant tumor cells, potentially increasing the efficacy of existing therapies[10]. The study explores miR-21's role in cancer hallmarks, thereby contributing to lung cancer progression. It also explores the potential of targeting these hallmarks, specifically through miR-21 downregulation to develop more effective therapies with fewer side effects.

Lung Cancer: Epidemiology and Pathology

Lung cancer is a heterogeneous disease, exhibiting diverse subtypes with distinct characteristics. Despite accounting for only 15% of new cancers diagnosed, it is the deadliest in the United States, accounting for ~28% of deaths in 2010[22]. In 2020, Canada paints a grim picture as the most diagnosed and leading cause of mortality due to lung cancer[35]. Understanding the molecular basis of this variability is crucial, as it may be attributed to different types of lung-lining cells or distinct cellular alterations within the same target cells[22]. NSCLC makes up to 80-85% with various subtypes including adenocarcinoma, squamous carcinoma, large-cell carcinoma and bronchoalveolar lung cancer. Within NSCLC, different genetic mutations are associated with specific subtypes. Small cell lung cancer (SCLC) makes up to 15-20% and has distinct molecular characteristics compared to NSCLC.

Tobacco smoke is responsible for 85% of lung cancers and 15 to 25% occur in people who have never smoked or very little. These original distinctions are related to variations in the molecular-level alterations that tumors undergo. Mutations in the *RGS17*, a G-protein family gene, have been an important cause of familial lung cancer but not sporadic lung cancer. In terms of epidemiology, clinical manifestation and molecular pathology, lung malignancies in non-smokers and smokers are not the same. Never-smoking lung cancer primarily affects women and people from East Asian backgrounds. It acquired mutations in the epidermal growth factor receptor (EGFR), making it highly susceptible to EGFR-targeted treatments. This type of cancer targets the distal airways.

It has been suggested that there may be a connection between head and neck cancer and uterine cervical cancer with the human papillomavirus (HPV), a recognized human carcinogen[22]. Currently, available treatments are radiotherapy, chemotherapy, surgery (for both stage I and stage II tumors) and targeted therapy[6]. Despite treatment advancements, the 5-year survival rate for lung cancer remains low at around 16%. The primary cause of death for about 70% of patients with advanced-stage lung cancer who were diagnosed within 18 months of their illness was due to metastatic spread. Therefore, developing novel strategies to prevent lung cancer cells from metastasizing is important, as this could aid in the treatment of patients in clinical settings[58]. The mutation identification in the various genes can lead to effective treatments, as these therapies are only applicable to a small subset of populations. This necessitates devising treatment for the remaining patients[6].

Tobacco smoke contains toxins that affect both the central and peripheral compartments. 85% of NSCLC and 98% of SCLC are caused by smoking. Among these carcinogens, polycyclic aromatic hydrocarbons (PAHs) and nicotine-derived nitroaminoketone are the main contributors causing genetic alterations by forming DNA adducts[16]. Oxidative lesions like 8-oxo guanine also occur under the influence of tobacco carcinogens. Several variants in cytochrome P450 1A1 gene and GSTM1 homozygous deletion have been linked with the risk of lung cancer[43]. Genes of the Myc family are overexpressed and may be amplified in both SCLC and NSCLC, while the *K-ras* oncogene is exclusively mutated in 30% of NSCLCs. TP53 and RB are altered in SCLC, with over 90% of cases showing mutations in TP53 and inactivation of the RB gene[29].

miR-21: A microRNA with oncogenic potential

miR-21 was the first mammalian small regulatory RNA that contributes to cancer, cardiovascular diseases, inflammation etc.[21] In homo sapiens, it resides on the 17th chromosome (17q.23.1), specifically within the eleventh intron of the transmembrane protein-49 (TMEM49). Despite their overlap and shared transcriptional direction, miR-21 possesses independent promoter regions and terminates with its poly(A)tail[45]. miRNA biogenesis begins with RNA polymerase II/III processing, which can occur post- or co-transcriptionally. It can be divided into intronic and intergenic miRNAs. Intronic miRNAs originate in introns and, to a lesser extent, from exons of protein-coding genes. Intergenic miRNAs are separately transcribed from host genes and are regulated by their promoters[19].

The canonical pathway begins with pri-miRNAs being transcribed from their genes. A microprocessor complex, consisting of the RNA binding protein DiGeorge Syndrome Critical Region 8 (DGCR8) and a ribonuclease III enzyme, Drosha, cleaves the pri-miRNAs into pre-miRNAs. DGCR8 identifies specific sequences, N6-methyl adenylated GGAC and other motifs, in pri-miRNA, while Drosha cuts the pri-miRNA duplex at the base of the hairpin structure[7]. This creates a 2 nt 3′overhang on pre-miRNA, transported by XPO5/RanGTP complex to the cytoplasm, processed by Dicer to remove the terminal loop, forming a mature miRNA duplex. The directionality of the miRNA strand determines its name, with 5p originating from the 5' end and 3p from the 3' end of the hairpin[40].

The miR-21 gene transcribes and produces 3433-nts long pri-miR-21, This pri-miR-21 is then cleaved into a shorter precursor molecule, 72-nts long pre-miR-21. Finally, it is processed to generate the mature miR-21-5p and miR-21-3p with 21 and 20 nts respectively[12].

miR-21 exhibits abnormal expression in numerous cancers such as glioma, lung cancer and breast cancer. Recent research suggests that miR-21 is an important biomarker for poor prognosis due to its association with disease incidence[55]. It exerts its influence by modulating the expression of a range of genes crucial for cancer initiation, progression and metastasis including phosphatase and tensin homolog (PTEN), programmed cell death protein 4 (PDCD4), purinergic receptor P2X7 and phosphoinositide-3-kinases (PI3K)[61]. The signal transducer and activator of transcription 3 (STAT3) and nuclear factor kappa-light-chain-enhancer of activated B cells (NF-kB) are constantly active in several malignancies. They play an essential role in maintaining cell proliferation, invasion, apoptosis and tumorigenesis and control miR-21 expression.

Nearly 30 to 40% of patients with lung cancer experience a devastating complication of bone metastases. Numerous studies have established a connection between miRNAs to bone metastasis of lung cancer[39]. Another study revealed that miR-21 suppresses apoptosis and promotes cell proliferation in H2170 NSCLC tumors[13]. In pancreatic ductal adenocarcinoma (PDAC), it was observed that when miR-21 was inhibited, transgenic *KRAS*-(G12D)/Trp53 null/Pdx1-cre (KPC) cell lines showed reduced proliferation, migration and invasion compared to wild-type pancreatic epithelial cells, suggesting that miR-21 mediates disease progression and metastasis[5]. In the Fisher rat thyroid cell line (FRTL-5), miR-21 was triggered by a specific hormone, hinting at a potential role in normal cellular development and specialization. This suggests that while excessive miR-21 may aid in cancer development, controlled levels might be required for normal growth and differentiation[8].

miR-21: Orchestrating hallmarks of lung cancer

(i) Sustaining proliferative signalling: Cancer cells undergo uncontrolled cell division and these hallmarks arise from their ability to continuously activate intracellular signaling pathways, typically involving the binding of tyrosine kinase domains[14]. EGFR is a key driver of cancer promoting DNA synthesis, cell cycle, proliferation etc.[26] Mutations in EGFR, anaplastic lymphoma kinase (ALK), or c-ras oncogene 1 (ROS1) are the common genetic alterations found in NSCLC patients. It is a member of the erbB family of receptor tyrosine kinases that consists of erbB1 (also known as EGFR), erbB2 (HER2), erbB3 and erbB4. In 43-89% of NSCLC patients, intracellular EGFR mutations or overexpression have been observed.

Studies have shown that a quarter of NSCLC patients had EGFR tyrosine kinase domain mutations, often accompanied by increased EGFR expression. The majority of EGFR domain mutations are found in exon 19 as short in-frame deletions or in exon 21 as point mutations, with the L858R mutation (arginine replacing leucine at codon 858)[4].

EGFR-mutated lung cancers are known for their fast growth and spread, exhibiting significantly higher miR-21 levels[44]. In NSCLC EGFR-mutant cell lines, miR-21 reduced PTEN expression promoting lung cancer[48]. Consistently, miR-21 overexpression decreased the effectiveness of gefitinib (first-line treatment for the patient harboring an activating EGFR mutation), by inhibiting PTEN and triggering AKT/ERK signaling[38].

Altered levels of miR-21 can influence PTEN expression and its related pathways which are important in regulating cancer cell behavior including invasion, migration and cell proliferation[30]. PTEN was the first identified tumor suppressor gene[57] that functions as a dual specialty phosphatase. As a direct product of phosphatidylinositol-3-kinase (PI3K), this phosphatase is a crucial regulator of cell proliferation, differentiation, growth and death. miR-21 binds to the 3′-UTR of PTEN mRNA to downregulate PTEN post-transcriptionally[60]. miR-21 inhibits the Ras/MEK/ERK pathway's negative regulators which in turn promote tumorigenesis[15]. miR-21 also exerts its functions by PTEN downregulation and cytokine signaling 1 and 6 (SOCS1/6) suppression, which alters the cell growth of NSCLC cells[36,56]. Collagen type I (Col-1) stimulates miR-21 levels which can lead to a loss of polarity in epithelial cells and can promote tumor growth[24].

(ii) Evading growth suppressors: Evading growth suppressors are the bypass or inactivation of proteins that restrict cell division and promote cell cycle arrest or apoptosis. Well-known examples of growth suppressor genes include p53 and retinoblastoma (Rb). Studies have demonstrated that knockout of p53 and Rb in mice can lead to the formation of SCLC[31]. This finding aligns with observations from human SCLC patients where whole-genome sequencing of tumor tissue samples revealed p53 and Rb gene deletion[11]. miR-21 was shown to be elevated in NSCLC with specific p53 mutations (R175H and R248Q). Higher levels of p53 mutations and miR-21 were associated with poorer survival outcomes[37]. Notably, miR-21 downregulates *PTEN* and tropomyosin 1 (*TPM1*), potentially contributing to cancer[8].

(iii) Ability to evade apoptosis: Evasion of apoptosis is a key hallmark of cancer cells to survive despite apoptotic signals[14]. Cancer cells can evade apoptosis by downregulating death receptors like the Fas receptor on the surface or upregulating decoy receptors that do not transmit apoptotic signals. They may also produce proteins that inhibit the death-inducing signaling complex (DISC) formation, thereby blocking the extrinsic apoptotic pathway[1]. p53 is a crucial gene that helps to trigger apoptosis when DNA is damaged. Mutated TP53 results in loss or

malfunction of p53, are the most common alterations in human cancer[53].

Foxo3a induced cell death in NSCLC by inhibiting miR-21 expression[54]. miR-21 regulates the AKT/P-AKT/cleavage-caspase 3 /MMP-2/MMP-9 signaling pathway and inhibits apoptosis while promoting the proliferation of lung cancer cells[25]. miR-21 blocked apoptosis in NSCLC xenografts by counteracting the Ras/MEK/ERK pathway inhibition[15].

(iv) Limitless replicative potential: Normal cells have a limited lifespan (mortal) but cancer cells exhibit limitless replicative potential, effectively achieving immortality. This distinction hinges on the activity of telomeres and their associated enzyme, telomerase. Telomerase, a specialized DNA polymerase, adds repetitive sequences (telomere repeats) to the ends of chromosomes (telomeric DNA). This process enables cancer cells to undergo numerous cell divisions without experiencing critical telomere shortening, a hallmark of cellular senescence in normal cells. Notably, telomerase activity is typically absent in most non-cancerous cells, leading to their inevitable replicative senescence[14].

(v) Sustained angiogenesis: Angiogenesis is a key process in tumor growth, as it provides tumors with the necessary blood supply, oxygen and nutrients leading to epithelial-mesenchymal transition (EMT)[33]. Cancer cells secrete vascular endothelial growth factor (VEGF), fibroblast growth factor 2(FGF-2) and epithelial growth factor (EGF) necessary for angiogenesis. These factors accelerate lung cancer development by stimulating the growth of new blood vessels. Exosomal miR-21 from cigarette smoke extract (CSE)-transformed human bronchial epithelial (HBE) cells activates STAT3 which increases VEGF-promoting angiogenesis[27].

(vi) Invasion and metastasis: Zhu et al[63] observed that miR-21 downregulation MDA-MB-231 cells showed a significant reduction in invasion and lung metastasis. Direct targets of miR-21 were identified as PDCD4 and mapsin, implicated in cell invasion and metastasis. The researchers investigated the regulatory role of STAT3 in brain metastasis-initiating cells (BMICs) using patient-derived stem cells from lung-to-brain metastasis. By targeting miR-21 and knocking down STAT3, they demonstrated that both approaches resulted in a decrease in BMIC self-renewal and migration. The study suggests that STAT3 and miR-21 cooperate as regulators of stemness, migration and tumor initiation[49].

(vii) Avoiding/escaping immune destruction: Various immune cells of the hematopoietic system include B and T lymphocytes, monocytes, macrophages and dendritic cells, express miR-21. High miR-21 levels have been correlated with immune cell activation and tumor progression. This may be due to its ability to suppress tumor suppressor genes involved in the intrinsic apoptotic pathway triggered by death receptors, ultimately promoting cell death by necrosis[47]. miR-21 targets in lung cancer pathogenesis are illustrated in fig. 1.

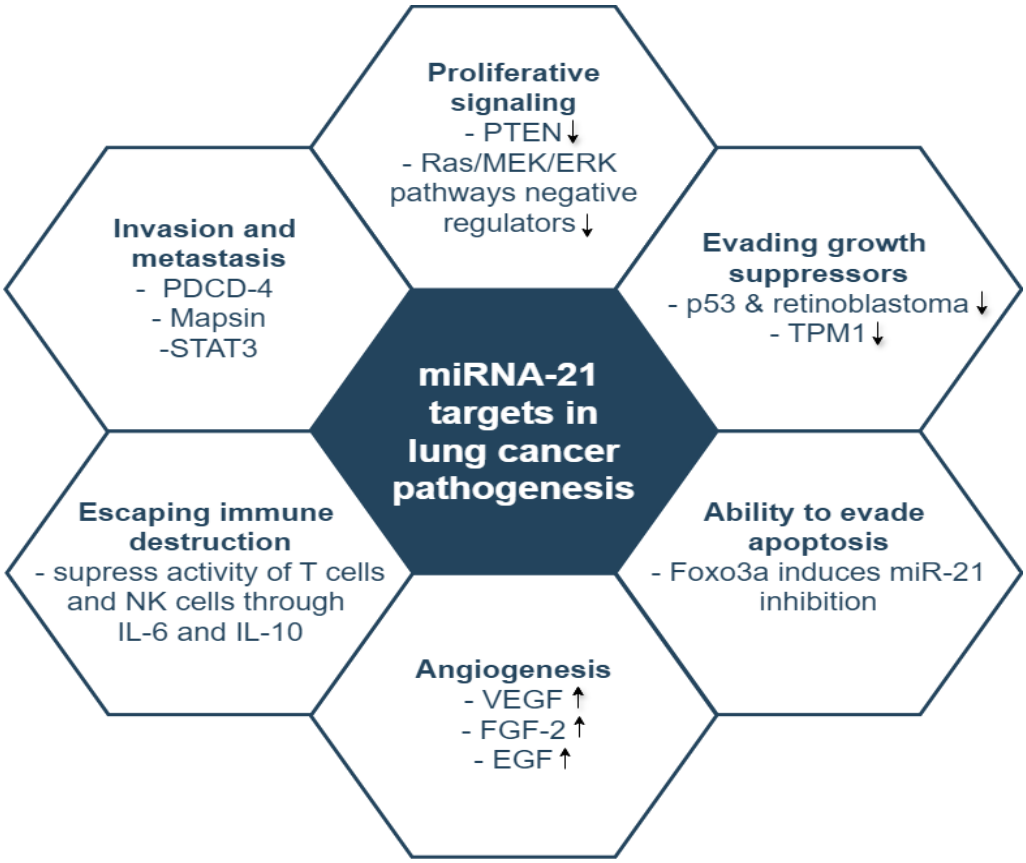

Figure 1: miRNA-21 targets in lung cancer pathogenesis

Table 1
Therapeutic potential of targeting miR-21 in lung cancer

Cell line/ animal model	Target genes	Hallmarks	Mechanism
Pc-9/GR	*PTEN*	Cell proliferation	Upregulation of PTEN expression restored gefitinib sensitivity and inactivated AKT/ERK pathway[48]
HUVEC	*STAT3*	Angiogenesis	Knockdown of STAT3 reduced miR-21 levels and decreased VEGF levels[28]
H1299	*IRF1*	Tumor progression	A hypoxic environment promotes macrophage M2 polarization through miR-21-rich exosomes, which suppress IRF1 expression, ultimately enhancing tumor progression[17]
A549	*HBP1*	Tumor invasion and migration	miR-21 promotes lung adenocarcinoma progression by targeting HBP1, enhancing cell growth, migration, invasion and EMT and could be a therapeutic target[50]
A549	*β-catenin*	Cell proliferation and survival	Reducing miR-21 levels decreases the levels of p-β-catenin, which in turn inhibits lung cancer growth[2]
A549, H460, H1299	*SMAD7*	Cell proliferation	miR-21-5p inhibitor suppressed lung cancer cell proliferation by inhibiting SMAD7[52]
A549	*NF-κB/PTEN*	Cell survival	Inhibition of NF-κB by RNAi mechanism decreases miR-21 expression[59]
NSCLC cell lines	*PTEN*	Cell growth and invasion	miR-21 exerts a suppressive effect on PTEN expression, resulting in the stimulation of growth and invasion in NSCLC[60]
NCI-H446, A549, NCI-H460	*hMSH2*	Cell proliferation	miR-21 expression is a key regulator of hMSH2 modulating cell cycle and proliferation[62]
A549	*PTEN*	Cell growth and metastasis	miR-21 mimics suppressed the expression of PTEN mRNA and protein. Conversely, introduction of anti-miR-21 reversed these effects[28]
PC9	*PTEN, PDCD4*	Apoptosis	miR-21 suppresses PTEN and PDCD4, leading to the activation of the PI3K/Akt signaling pathway[23]
A549, H1703	*CD36, PPARGC1B*	Cell growth and metastasis	Inhibition of miR-21 decreased cell growth, migration, intracellular lipid levels and CD36 protein expression. Also, PPARGC1B is a direct target of miR-21, where its downregulation reverses the inhibitory effect of miR-21 inhibitor[34]
H2170	*COX19*	Apoptosis	COX-19 plays a crucial role in miR-21-mediated apoptosis inhibition. Inhibition of COX-19 activity led to decreased cell proliferation by enhancing cell apoptosis[13]

miR-21 as a potential therapeutic target in lung cancer: The restoration or downregulation of specific miRNA expression levels holds potential as a therapeutic strategy for cancer treatment. Two primary approaches for developing miRNA-based therapeutics are: 1) miRNA mimics and 2) antogmiRs. miRNA mimics are synthetic molecules that mimic the function of endogenous miRNAs, potentially activating tumor-suppressor pathways. AntagomiRs are antisense oligonucleotides designed to inhibit the activity of oncogenic miRNAs, potentially suppressing tumor growth[20]. miR-21 has become a major target in miRNA therapeutics, which aims to harness the therapeutic potential of miRNAs by either replacing them using miRNA mimics or inhibiting their function using antimiRs[41]. Table 1 illustrates the therapeutic potential of targeting miR-21 in lung cancer.

Conclusion

Lung cancer remains a formidable foe in the battle against cancer, with conventional treatments like radiation and chemotherapy often associated with severe side effects. miRNAs, particularly miR-21, have emerged as pivotal regulators of gene expression and potential therapeutic targets. This review has highlighted the miR-21's multifaceted role in driving lung cancer progression by promoting cell growth, suppressing tumor suppressors, resisting cell death, enabling replicative immortality, inducing angiogenesis and promoting invasion and metastasis.

Moreover, miR-21's influence on immune regulation implies that inhibiting it could strengthen the immune

response against lung cancer cells, offering combined advantages of immune system activation and direct tumor reduction. The dual-targeting nature of miR-21 suggests that it could be a valuable therapeutic target, particularly for patients with few treatment options. Ultimately, targeting miR-21 could lead to developing more effective and less toxic therapies for lung cancer, improving patient outcomes.

Acknowledgement

The authors would like to thank the Management of Vellore Institute of Technology, Vellore for providing the required facilities to carry out the work.

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(Received 23rd September 2024, accepted 24th October 2024)