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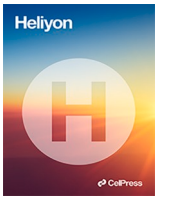

Review article

Nuclear epidermal growth factor receptor (nEGFR) in clinical treatment

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ABSTRACT

The epidermal growth factor receptor (EGFR) is a recognized target in tumor treatment. While there is significant focus on inhibiting membrane EGFR and its downstream signaling activation, the ectopic accumulation of EGFR, particularly nuclear EGFR (nEGFR), has been implicated in tumor-associated activities and associated with poor prognosis. Within the nucleus, nEGFR functions as a transcriptional regulator to modulate transcriptional landscape and exerts tyrosine kinase activity to phosphorylate nuclear proteins and subsequently influences DNA repair, cell cycle, proliferation, and resistance to radiotherapy and chemotherapy. The nuclear localization of EGFR involves the internalization, subcellular trafficking, and nuclear envelope shuttling of membrane EGFR. Given the challenges of delivering drugs to the nucleus for targeting nEGFR, understanding the molecules affecting the translocation process is crucial for novel insights. This review initially explores the association between nEGFR expression and clinical outcomes and then elucidates how nEGFR fulfills its regulatory role within the nucleus. Subsequently, the mechanisms governing EGFR nuclear translocation and potential therapeutic targets during this process are summarized, highlighting avenues to target nEGFR as an innovative strategy in tumor treatment.

1. Introduction

The epidermal growth factor receptor (EGFR) belongs to a family of membrane receptor tyrosine kinases (RTKs). When binding to ligands in the cell membrane, homodimerized or heterodimerized EGFR exhibits tyrosine activity, activating several cell signaling pathways, such as PI3K/Akt, Ras/Raf, and PLC-γ. The aberrant activation of membrane EGFR can occur due to different mechanisms, including gene mutations and amplifications in tumors, which may culminate in dysregulated gene transcriptional activities, ultimately fostering unlimited cell proliferation and unfavorable prognostic outcomes in tumor patients [1–5]. To counteract the tumorigenic effects of dysregulated activation of membrane EGFR, several therapeutic approaches have been developed, including EGFR tyrosine kinase inhibitors (TKI), monoclonal antibodies (mAbs), and vaccines like CIMAvax [6].

In addition to the critical role played in the cell membrane, EGFR is also located in other cell components, such as the nucleus, endosomes, and mitochondrion [7–9]. Ever since the pioneering work of Marti et al., in 1991, wherein EGFR was isolated from

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hepatocyte nuclei [7,], nuclear EGFR (nEGFR) has been substantiated across a diverse array of tissues including thyroid, placenta, bladder, lung, breast, and ovary [10–15]. Different from membrane EGFR, nEGFR mainly acts dual roles within the nuclear milieu, functioning as a transcriptional regulator to modulate transcriptional landscape [10,16], and exerting tyrosine kinase activity to phosphorylate nuclear proteins [17,18]. Clinically, nEGFR may be regarded as a negative prognostic indicator in tumors. Moreover, nEGFR has been considered to induce resistance against chemotherapy and radiotherapy [13,19]. Therefore, nEGFR-oriented targeted therapies should be considered. In this study, we summarized the clinical significance of nEGFR, the molecules and compounds regulating the nuclear translocation of EGFR, and nEGFR's biological functions, which may provide new insights into nEGFR targeting therapies.

2. Search strategy and selection criteria

This review is primarily based on PubMed using the terms "nuclear epidermal growth factor receptor," "nuclear EGFR," and "nEGFR". We excluded papers that were unrelated or lacked research on specific mechanisms of nEGFR. For clinical relevance, we summarized studies on nEGFR published between 2013 and 2024. As for the nuclear transportation and biological functions of nEGFR, biases may exist due to immature techniques in some early papers. Only articles published in English were included.

3. The clinical association between nuclear EGFR and tumors

In clinical studies, nEGFR's impact on cancer prognosis is nuanced and multifaceted, with both positive and negative associations observed across various cancer types (Table 1). On one hand, since membrane EGFR has been proved not prognostic for EGFR-targeting therapy, such as cetuximab, nEGFR shows its prognostic potential as an alternative [20]. Most researchers have found that overregulation of nEGFR or nEGFR gene signature is associated with worse prognosis and higher tumor status in non-small cell lung carcinoma (NSCLC), melanoma, head and neck squamous cell carcinoma, rectal cancer, and oral squamous cell carcinoma [3,4, 21–23]. Besides, nEGFR accumulation was associated with poor prognosis after treatment. For instance, Yang et al. acquired tumor samples from 172 rectal cancer patients who underwent neoadjuvant concurrent chemoradiotherapy [22]. It was reported that both advanced pre-treatment and post-treatment tumor stages were correlated with overexpression of nEGFR (T3 *vs.* T1-2, $p = 0.017$; and T4 *vs.* T1-2, $p < 0.001$ respectively) defined by immunohistochemistry analysis.

On the other hand, in clear cell renal cell carcinomas (CCRCCs), nEGFR appears to indicate better cancer status and a favorable prognosis [25]. What's more, immunohistochemical staining of 502 biopsies across 27 tumor types revealed that nEGFR accumulation was associated with low T stage [24], which suggests that nEGFR's tumor-promoting functions may predominantly operate in the early stages of carcinogenesis.

In addition to prognostic biomarkers, nEGFR serves as an indicator for tumorigenesis. For example, Tarle et al. reported that with a median follow-up time of 5.3 years, oral leukoplakia and oral erythroplakia patients with strong nEGFR staining faced an 8.4-fold ($p = 0.001$) higher risk of developing oral squamous cell carcinoma [26,]. Likewise, the nEGFR levels were found with higher frequency among laryngeal squamous cell carcinoma patients compared with patients with laryngeal dysplasia and polyps ($p < 0.001$) [21].

Table 1
Clinical research associated with nEGFR level.

Authors	Year	Pathology	Method	Patients with positive nEGFR expression	Prognosis	Clinical Characteristics: HR (95 % CI)	*p*-value	Other characteristics
Traynor AM et al. [3]	2013	NSCLC	IHC	23/88	Worse	PFS: 1.89(1.15–3.10); OS: 1.83(1.12–2.99)	PFS: 0.011; OS: 0.014	Higher disease stage
Katunarić M et al. [4]	2014	Melanoma	IHC and FISH	71/106	Worse	OS: 3.06(1.19–7.87)	<0.05	Higher mEGFR level
Yang CC et al. [22]	2019	Rectal cancer	IHC	37/172 (nEGFR overexpression)	Worse	DSS: 2.42(1.29–4.54); LRFS: 3.03(1.22–7.28)	DSS: 0.006; LRFS: 0.016	Higher T stage
Yan G et al. [24]	2019	27 tumor types	IHC	163/319	Better	T stage	0.004	Higher mcEGFR level
Marijić B et al. [21]	2021	HNSCC	IHC and FISH	39/42	Worse	OS: NR	0.025	Higher mEGFR level
Muroni MR et al. [25]	2021	CCRCC	IHC	11/57	Better	OS: NR	0.030	–
Tarle M et al. [23]	2023	OSCC	IHC	30/52 (nEGFR ++/+++)	Worse	OS: NR	0.004	More alcohol abuse; smoking

Other characteristics refer to features positively associated with the level of nEGFR. Abbreviations: NSCLC, non-small cell lung cancinoma; HNSCC, head and neck squamous cell carcinoma; CCRCC, clear cell renal cell carcinoma; OSCC, oral squamous cell carcinoma; IHC, immunohistochemistry; FISH, fluorescence in situ hybridization; nEGFR, nuclear epidermal growth factor receptor; HR, hazard ratio; PFS, progression-free survival; OS, overall survival; DSS, disease-specific survival; LRFS, local-recurrence free survival; NR, not reported; mEGFR, membrane epidermal growth factor receptor; mcEGFR, membrane-cytoplasmic epidermal growth factor receptor.

4. EGFR biological functions and its regulation in the nucleus in tumors

nEGFR can form complexes with other transcriptional factors and regulate transcription through binding to promoters of certain genes, like transactivation factors, and phosphorylates downstream nuclear proteins, which result in dysregulated cell cycle, DNA repair, and cell survival (Fig. 1). Interfering nEGFR kinase substrates and transcriptomic regulation functions may counteract the tumorigenic activity.

4.1. EGFR acts as a transcriptome regulator in the nucleus

nEGFR regulates transcription like transactivation factors; however, it needs collaboration with other transcription factors, and forms complexes to make up for its lack of a DNA-binding domain [10]. The proline-rich sequence in the C-terminal tail of EGFR was identified as the transactivation domain [10].

Lin et al. first reported that nEGFR fused to the GAL4 DNA-binding domains strongly activated the expression of a reporter gene [10]. In the nucleus, nEGFR forms complexes with various transcriptional cofactors, like RHA, E2F1, and STAT3. Functionally, those transcriptional factors can recognize AT-rich sequences of gene promoter and assist nEGFR to execute transactivation, but which domain of nEGFR interacts with those transcriptional factors remains to be further explored [27].

4.1.1. nEGFR-STATs complexes

nEGFR-STAT3 complex engaged the promoter region of COX-2, thereby potentiating inflammatory pathways [28]. Furthermore, the same complex lends itself to the heightened expression of STAT1, further intensifying the inflammatory response [29]. It has also come to light that nEGFR-STAT3 binds to the LIFR promoter, thereby fostering neuroendocrine differentiation and glycolysis in prostate cancer [30]. Nuclear accumulation of EGFR and STAT3 are enhanced by various factors, including LMP1 and IGFBP2, and the nEGFR-STAT3 transcription complexes are more formed, promoting the expression of cyclin D1 and affecting the cell cycle [31,32]. Additionally, the phosphorylation of Fas at Y291 triggers the accumulation of phosphorylated EGFR and STAT3 within the nucleus, potentially promoting the formation of nEGFR-STAT3 complexes [33]. Besides, prostaglandin E2 (PGE2), a classic inflammatory mediator, enhanced the endocytosis of EGFR and this increased the formation of nEGFR-STAT3 complexes, thereby extending nEGFR's transcriptional activity for tumorigenic proteins [34]. In terms of compounds, dihydroconiferyl ferulate has been found to reduce nEGFR levels and induce apoptosis by inhibiting the nEGFR/STAT3/c-Myc pathway [35]. Plus, nEGFR also combines with STAT5, and

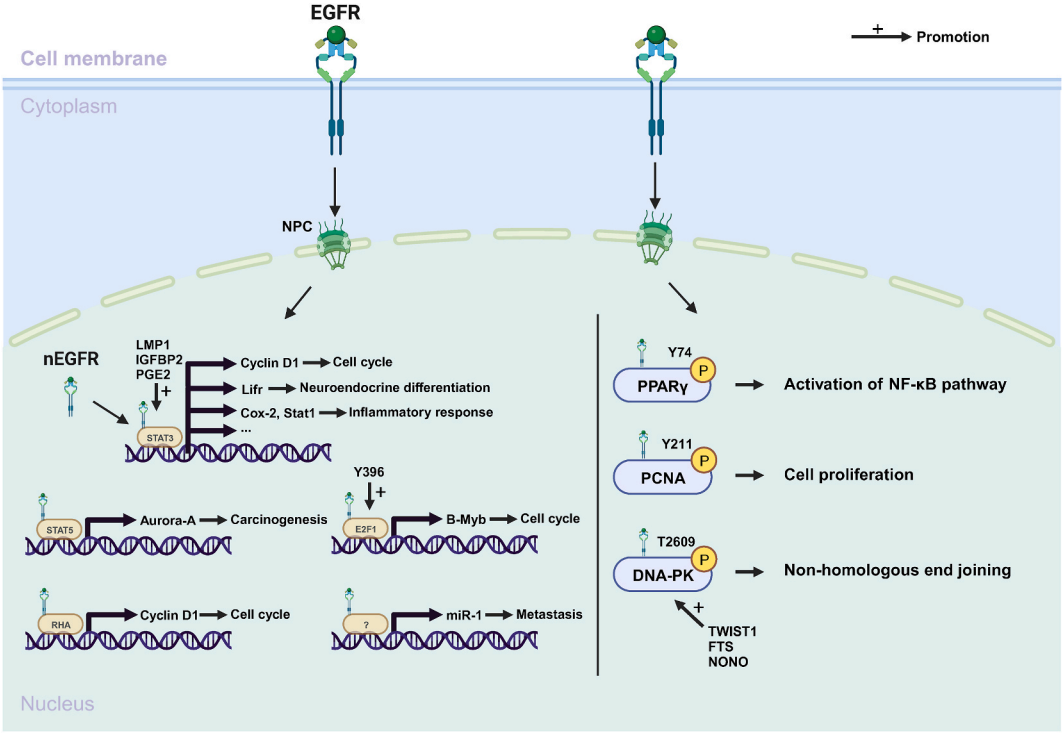

Fig. 1. Biological functions of EGFR in the nucleus. After membrane EGFR is transported through nuclear pore complexes (NPC) into the nucleus, it mainly exerts two types of biological function. (a). Nuclear EGFR (nEGFR) combines transcriptional factors, such as STAT3, STAT5, E2F1, and then forms transcriptional complexes, which bind to promoters of certain genes and affect cell cycle, tumorigenic activities, and inflammatory response. (b). Nuclear EGFR acts as a kinase to phosphorylate nuclear proteins, like PPARγ, PCNA, and DNA-PK, promoting cell proliferation, DNA repairs, and other pathways.

the nEGFR-STAT5 complex activates the expression of Aurora-A, a factor implicated in carcinogenesis and chromosome instability [36, 37]. What's more, nEGFR-STAT5 complexes can also confer resistance to osimertinib through increasing AURKA expression [38].

4.1.2. Other nEGFR complexes

First, by engaging with RHA, nEGFR takes an active role in fostering the expression of cyclin D1, a pivotal orchestrator of cell cycle progression [27]. Second, nEGFR forms complexes with E2F1 that modulate the transcription of B-Myb, fostering a consequential upregulation of its expression, thereby profoundly influencing the dynamics of the cell cycle [39]. In non-tumor disease, for example, diabetes, attenuations of the interaction between E2F1 and EGFR in the nucleus via Y396, a novel rhynchophylline analog, lead to downregulated NOX4 and rescue endothelial dysfunction [40].

Moreover, nEGFR plays a role as a transcriptional repressor for miR-1, thereby hastening bone metastasis in prostate cancer [41]. Nevertheless, which molecule assists nEGFR in binding to the stem-loop promoter of miR-1 is unclear.

4.2. EGFR acts as a kinase in the nucleus

Conventionally, membrane EGFR exerts auto-phosphorylation after ligand occupation or being self-activated when mutated, then downstream signaling pathways such as RAS/RAF/MEK/Erk, PI3K/Akt, and JAK/Stat pathways are subsequently phosphorylated and activated when signaling proteins bind to phosphorylated tyrosine residues, regulating cell proliferation, survival, differentiation, and etc. Within the nucleus, nEGFR also manifests kinase activity and phosphorylates key nuclear proteins that wield significant influence over cellular processes, rather than auto-phosphorylation and triggers for classical cascade signaling.

As previously reported, proliferating cell nuclear antigen (PCNA) is stabilized when undergoing phosphorylation at Tyr-211 through nEGFR, contributing to the promotion of cell proliferation [17]. In addition, nEGFR was reported to phosphorylate peroxisome proliferator-activated receptor gamma (PPARγ) at Tyr-74, facilitating its degradation and activation of nuclear factor kappa B (NF-κB) [18].

DNA-dependent protein kinase (DNA-PK) is a critical component in DNA repair, which participates in non-homologous end joining (NHEJ). The activation of DNA-PK by nEGFR is regulated by various factors and is closely related to resistance to therapy-induced double-strand break (DSB). Accumulation of nEGFR correlates with elevated phosphorylation of DNA-PK at T2609, associated with

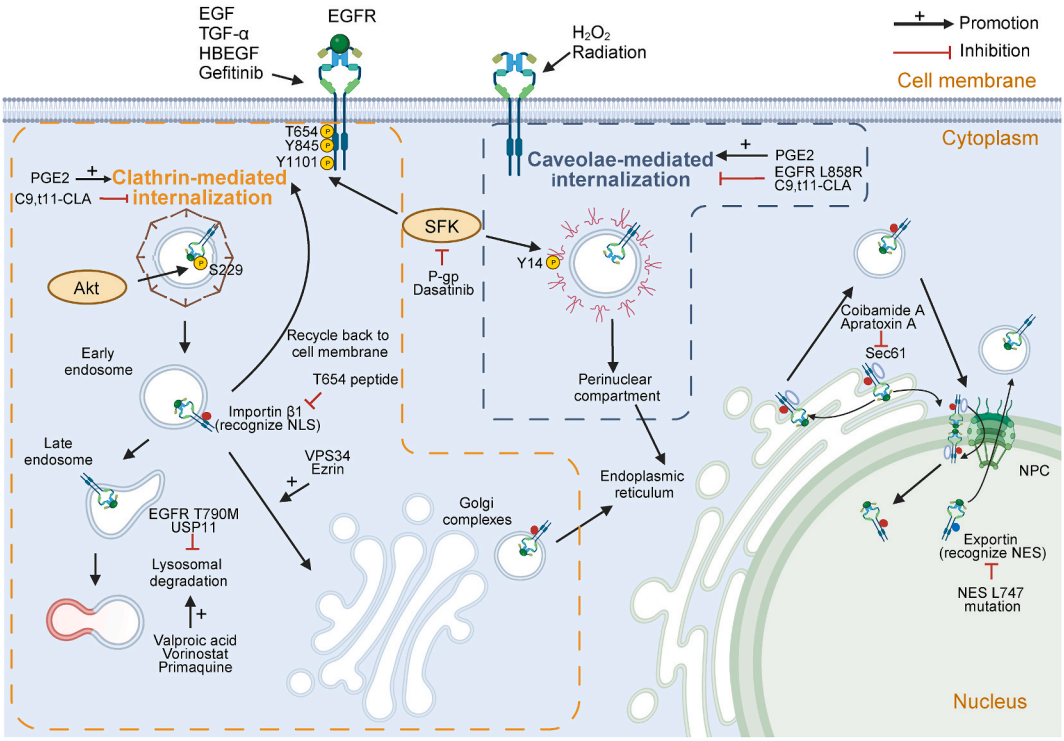

Fig. 2. Translocation of EGFR from cell membrane to nucleus. The trafficking of the EGFR involves both ligand-dependent and ligand-independent pathways mediated by clathrin and caveolae. In the clathrin-mediated pathway, EGFR can be recycled or undergo lysosomal degradation. After transiting through Golgi complexes and the endoplasmic reticulum, EGFR can enter the nucleus directly through nuclear pore complexes (NPC) or by returning to the cytosol before nuclear entry, facilitated by importin β1 and Sec61. Meanwhile, the caveolae-mediated pathway allows more efficient transport of EGFR to the perinuclear compartment and subsequent nuclear entry. The intricate regulation of EGFR phosphorylation sites, mutations, and the involvement of specific molecules and drugs finely orchestrate its nuclear transport, contributing to the precise control of cellular responses.

the activation of its kinase activity, after ionizing radiation [42]. Furthermore, it was revealed that both wild-type EGFR and EGFRvIII, not EGFR L858R mutant, bind to the catalytic subunit of DNA-PK (DNA-PKcs) in the nucleus [43]. It's deducible that nEGFR may mediate DNA-PK phosphorylation at T2609 and enhance the kinase activity of DNA-PK to form radiation resistance.

Several proteins participate in the interactions between nEGFR and DNA-PK and can be taken as potential targets for therapies. First, in hypoxic conditions, high levels of Twist1 increase the nuclear localization of EGFR and DNA-PKcs, enhancing DNA repair and radioresistance [44]. Second, upon radiation, the overexpression of FTS and EGFR in both the cytoplasm and nucleus was induced. Silencing of FTS destabilizes EGFR and DNA-PK, ultimately enhancing the formation of DSBs [45]. What's more, NONO, a key factor, forms liquid-liquid phase separation droplets with DNA-PK and EGFR, enhancing DNA-PK phosphorylation at T2609 and promoting DNA repair [46].

In conclusion, direct interference of proteins phosphorylated by nEGFR and blockade of the formation of nEGFR transactivation complexes may enrich tumor therapies. However, further investigations are required for the therapeutic effects of targeting other nEGFR complexes and more regulated proteins, like PCNA.

4.3. Other functions of EGFR in the nucleus

In addition to transactivation and kinase activity, there are some other mechanisms in which nEGFR indirectly regulates cellular processes. First, within the context of DNA damage repair, nEGFR exerts regulatory control over TIP60 acetylase activity which enhances the activation of ATM, a key role in the DNA repair process [47]. Other than that, nEGFR blocks PNPase, a negative regulator of transcription, and thus stabilizes mRNAs associated with the Warburg effect via forming complexes with mRNAs, effectively endowing cells with heightened radiation resistance [48]. As an inverse feedback, promyelocytic leukemia protein isoform IV is recruited to chromatin by nEGFR, which effectively diminishes promoter acetylation levels and suppresses the transcription of target genes of nEGFR [49].

5. Nuclear translocation of EGFR

The nuclear translocation of EGFR is a complex process, with the help of several certain molecules and modifications, which are potential targets for blocking the formation of nEGFR (Fig. 2)

5.1. Mechanisms of EGFR nuclear translocation

The process of EGFR's nuclear translocation can be divided into two major procedures: induction and trafficking. The nuclear translocation of EGFR begins with the endocytosis of membrane-bound EGFR via two distinct pathways: clathrin-mediated and caveolae-mediated. This leads to the formation of early endosomes, which undergo endosomal sorting through the Golgi complex (GC) and endoplasmic reticulum (ER). Some of these endosomes are recycled back to the cell membrane or targeted for degradation by lysosomes and proteasomes. Ultimately, EGFR-containing endosomes are transported into the nucleus through the nuclear pore complex (NPC).

Nuclear translocation of EGFR is initiated either through a ligand-dependent or a ligand-independent mechanism, namely the induction phase [13,50]. In the former scenario, ligand engagement, such as with epidermal growth factor (EGF), transforming growth factor-α, and heparin-binding EGF-like growth factor, triggers clathrin-dependent internalization, leading to the formation of coated pits that subsequently enter early endosomes within the cytosol [50,51]. Somewhat differently, certain instances of ligand-independent internalization, such as radiation-induced endocytosis, operate via a caveolae-mediated pathway [52]. Because of the protection of caveolae, endosomes containing EGFR are carried to the perinuclear compartment, prone to continued nuclear translocation rather than lysosomal degradation [53]. This protection by caveolae may be a consequence to counter imposed stress by prolonging nuclear EGFR signaling. Recently, Kim et al. revealed that inhibition of clathrin-mediated internalization combined with gefitinib induced apoptosis of gefitinib-refractory cell lines, which own relatively high IC50 concentration of gefitinib [54]. p38-mediated phosphorylation of unliganded EGFR monomers induces clathrin-mediated endocytosis [55]. Increased phosphorylation of STAT3, a downstream target of p38, in gefitinib-resistant cells suggests that inhibiting clathrin-mediated rather than caveolae-mediated endocytosis may restore gefitinib sensitivity [54]. This highlights the need to identify which pathway is involved in EGFR endocytosis in clinical therapy.

Next, clathrin-mediated endosomes can be degraded by lysosomes. The remaining endosomes and caveolae-mediated endosomes are ferried to GC and then undergo retrograde transport to ER during cytoplasmic trafficking [56]. The subsequent stages of translocation through the outer nuclear membrane are conducted in two routes. One route is that EGFR can be directly transported through NPC into the nucleus, because of the continuity between ER and nuclear membrane. Another route is that EGFR is released back into the cytoplasm and then subsequently transported through NPC. Recent findings have introduced a novel paradigm referred to as nucleus-associated endosomes, wherein some endosomes directly fuse with the outer nuclear membrane, bypassing the shuttling between ER and GC [57]. In the process of releasing EGFR from membranes, Sec61 translocon is recognized to play a dual role, not only in ferrying EGFR from the ER to the cytoplasm but also from the inner nuclear membrane to the nucleoplasm [58,59]. Next, to enter the nucleus, EGFR's distinct tripartite nuclear localization sequence (NLS) motif, RRRHIVRKRTLRR (amino acids 645–657), is essential [60], facilitating interaction with importin β1 and orchestrating translocation from the outer nuclear membrane to the inner nuclear membrane [59,61].

The genetic status of EGFR has complex effects on nEGFR translocation in cancer. One notable mutation, L858R, has been

surprisingly reported to inhibit EGFR internalization induced by radiation, potentially increasing the sensitivity of tumors to radiotherapy [62]. Though the interaction between EGFR and actin was reported to be associated with EGFR/EGF transport to lysosomes, it was found that EGFR interacts with cytoskeletal components only in the nucleus of EGFR-mutant cell line H1975, which conferred EGFR-TKI resistance [63,64].

5.2. Regulations and potential targets of nuclear transport of EGFR

Canonical targeting therapies aim at blocking membrane-bound EGFR signaling, but it's tough to deliver targeted drugs when EGFR is transported into the nucleus. Targeting essential modifications of EGFR in nuclear transport, the molecules participating in the process of EGFR nuclear translocation and nuclear transport signals may provide new approaches.

5.2.1. Targeting nuclear transport signals of EGFR

Nuclear transport signals, including nuclear export sequences (NES) and NLS, which have been previously mentioned, are crucial for EGFR trafficking into and out of the nucleus, thereby influencing the accumulation of nEGFR. The L747 mutation of NES of EGFR retains EGFR in the nucleus and thus promotes its accumulation, which dictates tumorigenic outcomes [65]. Targeting EGFR's NLS is another approach. The T654 peptide, designed to interfere with EGFR phosphorylation at the critical T654 site within its NLS, has shown potential in promoting cancer cell death and inhibiting tumor growth [66]. This effect was also observed in xenograft mouse models, highlighting its therapeutic promise. However, a novel mechanism involving the direct transport of EGFR and EGFRvIII to the nucleus of other cells via extracellular vesicles (EVs) has recently been identified. Importantly, this process occurs independently of NLS which is typically required for the nuclear translocation of endogenous EGFR, which may impair NLS-targeting strategy [61].

5.2.2. Targeting modifications of EGFR

Notably, phosphorylation sites within the intricate landscape of EGFR's nuclear trafficking warrant particular attention in the pursuit of improved therapeutic interventions. The Src family kinase (SFK) emerges as a critical regulator of EGFR nuclear localization upon radiation stress. Mechanically, radiation-induced lipid products can activate Src kinase, thereby promoting EGFR transport and modulating radiosensitivity [67]. The activation of Src kinases culminates in the phosphorylation of caveolin-1 Y14 and EGFR Y845, which initiates the caveolae-mediated internalization of EGFR [52,68].

Apart from irradiation, some other factors can activate SFK to initiate subsequent EGFR internalization and nuclear translocation. For instance, Bowman-Birk proteinase can similarly activate Src kinase, thereby inducing EGFR phosphorylated at T654, correlated with EGFR nuclear accumulation [42]. Also, PGE2 has been revealed to bind to its receptor EP3, leading to the activation of Src family kinases and facilitating the nuclear translocation of EGFR [69]. What's more, Yes and Lyn kinases, members of SFK, impact EGFR nuclear translocation by phosphorylating residue Y1101, thereby conferring resistance upon cetuximab treatment [70]. Besides, Li et al. reported that inhibitors of Src kinases, may suppress EGFR nuclear translocation and retain membrane EGFR, restoring sensitivity to cetuximab [71]. Moreover, in multi-drug-resistant cells, overexpression of P-glycoprotein (P-gp) was identified to weaken DSB repair activity by inhibiting the Src/EGFR translocation cascade through an unknown mechanism [72].

Some other molecules also take part in the regulation of EGFR nuclear transport. For example, Akt-mediated phosphorylation of EGFR at S229 proves essential for nuclear transport and resistance to gefitinib [73]. Additionally, DUOX1, a member of the NADPH oxidase family, enhances the cysteine oxidation in EGFR when downregulated, which affects endosomal sorting in GC and promotes the nuclear localization of EGFR, contributing to tumorigenic activities [74].

5.2.3. Targeting molecules attributed to EGFR nuclear transport

In terms of the initiation of internalization of EGFR, one promising avenue involves disrupting lipid distribution on the cell membrane. Compounds like cis-9, trans-11 conjugated linoleic acid (c9, t11-CLA) have demonstrated the ability to prevent EGFR internalization by interacting with and destablizing lipid rafts [75]. By hindering EGFR's internalization, these compounds enhance the sensitivity of cancer cells to radiation therapy [75]. Additionally, PGE2 treatment was demonstrated to initiate EGFR endocytosis through both clathrin-dependent and caveolin-dependent ways, underlining its importance in EGFR nuclear transport in tumors [34].

Endosomal transport also should be focused on EGFR nuclear shuttling. For example, vacuolar sorting protein 34 (VPS34) emerges as a promoter of transport of EGFR-containing endosomes to GC and its inhibition leads to reduced shuttling of EGFR [76]. Ezrin, a scaffold protein that mediates EGFR trafficking from early endosomes to the nucleus, can be inhibited to reduce nEGFR levels [77]. Critically, this inhibition has synergized with erlotinib, an EGFR inhibitor, undermining cell viability and restoring erlotinib sensitivity, particularly in NSCLC cells. Additionally, hnRNP A3, a spliceosome component participating in RNA processing/splicing, was found to interact with nuclear EGFR, and its downregulation inhibited the nuclear accumulation of EGFR without affecting the total EGFR levels, suggesting a redistribution mechanism of EGFR that remains to be elucidated [78]. Inhibitors of Sec61, such as coibamide A and apratoxin A, can downregulate the expression of HER/ErbB family proteins and inhibit cell growth [79]. The Sec61 subunit, Sec61G, is co-amplified with EGFR in glioblastoma and promotes immune evasion, suggesting that Sec61 could be a potential target for nEGFR-targeted therapy [80]. However, it remains unclear whether specific Sec61 inhibitors exert anti-tumor effects by blocking EGFR nuclear translocation.

In the cytosol, EGFR-containing endosomes can also be degraded rather than transported to the nucleus. Therefore, targeting lysosomal degradation of EGFR has also shown considerable potential. Valproic acid, when combined with cisplatin and cetuximab, has demonstrated the ability to downregulate EGFR expression and prevent its nuclear translocation via enhancing proteasomal or lysosomal degradation [81,82]. Analogously, vorinostat, an epigenetic modifier, has been investigated for its ability to impair EGFR's

nuclear translocation by enhancing lysosomal degradation pathways, resulting in reduced nuclear EGFR levels and potentially curtailing its tumorigenic activity [83]. Primaquine, an anti-malarial drug, was reported to offer an intriguing approach to disrupting EGFR endosomal trafficking [84]. This disruption ultimately leads to the degradation of early endosomes containing EGFR, potentially limiting its availability for nuclear translocation [84]. Additionally, the depletion of USP11, a deubiquitinase that can deubiquitylate EGFR, promotes the degradation of EGFR, reducing nuclear EGFR levels and alleviating tumorigenic activities, including the epithelial-mesenchymal transition (EMT) [85].

5.2.4. Other regulation of nEGFR

Some researchers revealed certain approaches may affect therapy-resistance during treatment, but further mechanisms need to be illustrated. For example, to recover the sensitivity of tumor cells, monoclonal antibodies like nimotuzumab have been developed specifically to target nEGFR. It reduces nEGFR accumulation and phosphorylated DNA-PK, impairing DNA repair processes and enhancing radiotherapy efficacy [86]. Innovatively, nanoparticles with Chitosan-MA-TPGS polymer have been designed to encapsulate anti-tumor drugs like erlotinib and quercetin, decreasing nEGFR accumulation [87]. These nanoparticles have the potential to reverse resistance to erlotinib in specific cancer cell lines, providing a novel approach to combating therapeutic resistance [87]. Remarkably, in the context of HPV infections, certain HPV oncoproteins facilitate nEGFR formation, potentially improving the prognosis of lung adenocarcinoma patients who accepted cisplatin therapy [88].

6. Future challenges and prospects in nEGFR-Targeting drug delivery

As discussed, targeting nuclear translocation by inhibiting NLS, NES, and key molecules like Src kinase holds potential for future therapies. Also, molecules enhancing the degradation of EGFR offer another way to decrease the level of nEGFR. However, direct targeting of nEGFR has not been extensively explored in recent years. Drugs that inhibit nEGFR's C-terminal tail, which relates to transactivation function, may counter nEGFR's role in gene regulation. Furthermore, research is needed to identify the specific domain responsible for nEGFR's interaction with other transcription factors, offering new opportunities for drug development. Besides, the efficacy of EGFR-TKIs in blocking EGFR's kinase activity remains debatable.

For drug delivery, unlike therapies targeting membranous EGFR, drugs aimed at nEGFR must be transported into the nucleus. Nanoparticles offer a promising solution to this challenge. Various nanocarriers, such as liposomes, polymer micelles, and nanoshells, have been developed for clinical use [89]. Their efficiency can be enhanced by conjugating NLS to the carrier, adjusting carrier size for nuclear entry, and incorporating positively charged lipids or polymers [90].

7. Discussion

Similar to membrane-bound EGFR, the overexpression of nEGFR is closely correlated with malignancy and poor clinical outcomes in most cases. As shown above, nEGFR can serve as a bypass mechanism to make up for the deficiency of EGFR functions in membrane-EGFR targeted therapies, contributing to resistance against EGFR-TKIs like gefitinib and erlotinib [54,77]. In addition to functioning as a kinase like membrane EGFR, nEGFR uniquely acts as a transcription regulator in the nucleus, promoting the expression of tumor-related genes. Thus, targeting nEGFR by blocking its nuclear transport or reducing its oncogenic function presents a promising strategy for cancer therapy, particularly in cases where tumor resistance develops during treatment.

However, several critical questions and challenges remain to be addressed in this field of research. First, investigating the impact of various mutations on EGFR translocation is crucial for the development of precise and personalized cancer therapies. Second, it is

Table 2
Comparison of modifications and mutations in nEGFR and classical EGFR.

Authors	Year	Modification or mutation	Functions in nEGFR	Functions in classical EGFR
Dittmann, K. et al. [52]	2008	Y845-p	Initiation of caveolae-mediated internalization	Activation of kinase activity [68]
Dittmann, K. et al. [42]	2008	T654-p	Supporting EGFR/karyopherin α complexes for nuclear entry	Activation of kinase activity; Stabilization of EGFR [91]
Dittmann, K. et al. [92]	2010			
Huang, W.C. et al. [73]	2011	S229-p	Increased nuclear translocation (unclear mechanism)	Unclear
Lida, M. et al. [70]	2013	Y1101-p	Increased nuclear translocation (unclear mechanism)	Unclear
Saloura, V. et al. [93]	2017	L721-m1	Enhanced interaction with PCNA	Augmentation of other phosphorylation [93]
Chiu, H.C. et al. [63]	2012	T790M	Regulation of transcriptions of EGFR-mediated genes	Hindering binding of EGFR-TKI [94]
Nie, L. et al. [65]	2023	L747A; L747P; L747S	Decreased export of EGFR from the nucleus	EGFR-TKI resistance (unclear mechanism) [65]

Abbreviations: nEGFR, nuclear epidermal growth factor receptor; -p, phosphorylation; -m1, mono-methylation; PCNA, proliferating cell nuclear antigen; EGFR-TKI, epidermal growth factor receptor-tyrosine kinase inhibitor.

known that a few EGFR-containing endosomes also are recycled back to the membrane, and whether enhancement of this process may impede EGFR nuclear translocation remains to be discovered. Third, it is essential to figure out if the decrease of nEGFR accumulation is accompanied by an altered total or membrane level of EGFR and promotes classical EGFR tumorigenic activities. Last but not least, it is important to note that modifications and mutations critical for EGFR nuclear translocation often overlap with those essential for classical EGFR signaling pathways (Table 2). For instance, phosphorylation at Y845 and T654 contributes to the kinase activity of membrane-bound EGFR, with T654 also stabilizing EGFR from degradation [42,52,68,91,92]. Mono-methylation at L721 enhances nEGFR's interaction with PCNA while promoting further EGFR phosphorylation, thereby activating downstream pathways [93]. Notably, certain phosphorylation sites like S229 and Y1101 appear to be specific to EGFR nuclear translocation, and their role in classical EGFR signaling remains to be explored [70,73]. Additionally, mutations such as T790M and L747, commonly associated with EGFR-TKI resistance, have been found to increase nuclear accumulation and enhance nEGFR functions [63,65,94]. Therefore, it is worth investigating whether membrane-bound EGFR functions are also affected by these overlapping modifications and mutations. Finally, to develop targeted therapies for nEGFR, rigorous clinical trials are necessary to assess the safety and efficacy of drugs that specifically target cytoplasmic and membranous molecules involved in nEGFR formation and functions.

Besides, there are also some limitations worth noting. First, there are few solid clinical studies on the effect of nEGFR on prognosis. Furthermore, although some research employs multivariate analysis, critical factors such as tumor genotype and EGFR amplification status were not fully considered. Second, most clinical studies used a single antibody to stain both membranous-cytoplasmic and nuclear EGFR, which may reduce the accuracy in identifying specific locations. Given the significant tumor-promoting functions of EGFR in the nucleus, we expect more accurate and robust clinical research to unveil the relationship between nEGFR levels and prognosis.

In summary, nEGFR research holds significant promise, but further investigations and clinical validations are required to unlock its full potential as a prognostic marker and therapeutic target in cancer management.

CRediT authorship contribution statement

Junkan Zhu: Writing – review & editing, Writing – original draft, Visualization, Supervision, Investigation. **Zhiyao Wu:** Writing – review & editing, Writing – original draft, Supervision, Investigation. **Guangyao Shan:** Writing – review & editing, Supervision. **Yiwei Huang:** Writing – review & editing, Supervision. **Jiaqi Liang:** Writing – review & editing, Supervision, Conceptualization. **Cheng Zhan:** Writing – review & editing, Supervision, Conceptualization.

Ethics approval and consent to participate

Not applicable.

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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