BIOTECHNOLOGY

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Development of Targeted EGFR Degradation for Cancer Treatment

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Introduction. The main distinguishing hallmarks of cancer are self-sustaining growth signals, insensitivity to growth inhibition signals, tissue invasion and metastasis, unlimited ability to replicate, and prevention of cell death [1]. In the fight against cancer, two main principles of small molecule therapy have been developed: standard therapy and targeted therapy. Standard chemotherapeutic agents are cytotoxic because they kill cancer cells, while targeted chemotherapeutic agents are often cytostatic because they bind to tumor cells and block cell proliferation. Targeted cancer treatment requires reliable information about human genes and proteins; therefore, it became the cornerstone of precision medicine for almost three decades.

Transmembrane receptor tyrosine kinases (RTKs) control various signaling pathways that play a pivotal role in the regulation of cell proliferation, motility, survival, and cell death [2]. Mutations that disrupt the functions of the intracellular kinase domain of the epidermal growth factor receptor (EGFR) are often associated with the onset and progression of cancer (Fig. 1). Target-specific small molecules and neutralizing antibodies have been designed to inhibit proliferative phosphorylation in signaling pathways triggered by RTKs in cancer cells. Targeting the ATP binding site in RTK is an important issue in medicinal chemistry for the treatment of EGFR-associated cancer [3]. Tyrosine kinase inhibitors (TKIs) with reversible and irreversible mechanisms of action have been developed to inhibit the catalytic site, improving patient survival compared to platinum-based chemotherapy, the previous standard of care [4].

However, resistance mutations leading to cancer progression have been described that include not only mutations in EGFR, but also those located outside the receptor gene and affecting HER2/HER3 amplification, mesenchymal epithelial transition factor (MET) amplification, the phosphoinositide 3-kinase (PI3K) pathway, mitogen-activated RAS protein kinase pathway (MAPK), and modification of cell cycle genes. Thus, the bottleneck of inhibitory chemotherapy targeting EGFR is the acquisition of multiple mutations in the same tumor, leading to drug resistance and unexpected side effects that reduce the effectiveness of TKIs.

In recent years, promising approaches have been developed aimed at targeted protein degradation rather than inhibition of the catalytic site [5]. The popular method of proteolysis-targeted chimeras (PROTAC) is based on the use of heterobifunctional degraders containing two linked moieties, one of which binds to the protein of interest, and the other binds to the E3 ligase [6]. The E3 ligase-degrader-protein complex results in polyubiquitination of the target protein and its subsequent degradation by the ubiquitin-proteasome system, after which the proteolysis-targeting chimera is recycled to target another copy of the protein of interest.

We have developed an alternative method for targeted degradation of EGFR by polyfunctionalized heterocyclic compounds, namely 4-allyl-5-[2-(4'-alkoxyphenyl)quinolin-4-yl]-4H-1,2,4-triazole-3-thiols, which bind to the receptor and lead to cell detachment from the extracellular matrix [7]. In this review, we highlight the processes that occur when compounds bind to EGFR and ultimately lead to cancer cell death. We suggest that targeted protein degradation holds great promise for improving the treatment of EGFR-associated cancer by overcoming the shortcomings of current TKI-based therapies.

Targeting EGFR in cancer. Post-translational modifications and protein-protein interactions directly modulate EGFR signaling and trafficking. A clue to understanding the inhibition of the tyrosine kinase activity of EGFR by TKIs is the finding that the driving force that activates the ATP-binding site is the action of hydrogen peroxide (H_2O_2) that is generated during cognate ligand EGF binding to the receptor [8]. The binding of EGF to EGFR promotes the transformation of O_2 to H_2O_2 through the membrane-located NADPH oxidase Nox2; then, this reactive oxygen species reacts with Cys797, leading to the transition of the thiolate anion (Cys-S) to sulfenic acid (Cys-SOH), which is required for the activation of the ATP-binding site in the receptor [9].

Ligand-independent auto-phosphorylation of EGFR has been also descrybed in cells treated with small molecules. In particular, the action of 4-nitrobenzoxadiazole derivatives rely on the generation of H_2O_2 by cytoplasmic superoxide dismutase in cancer cells [10]. Overall, this finding suggests that the highly reactive hydrogen peroxide produced by various metabolic reactions may unpredictably increase phosphorylation flux in EGFR-driven pathways and thus reduce the therapeutic efficacy of TKIs [11]. Below, we describe the properties of new generation of small molecules that overcome the disadvantages of H_2O_2 producing molecules when binding to the tyrosine kinase domain of EGFR.
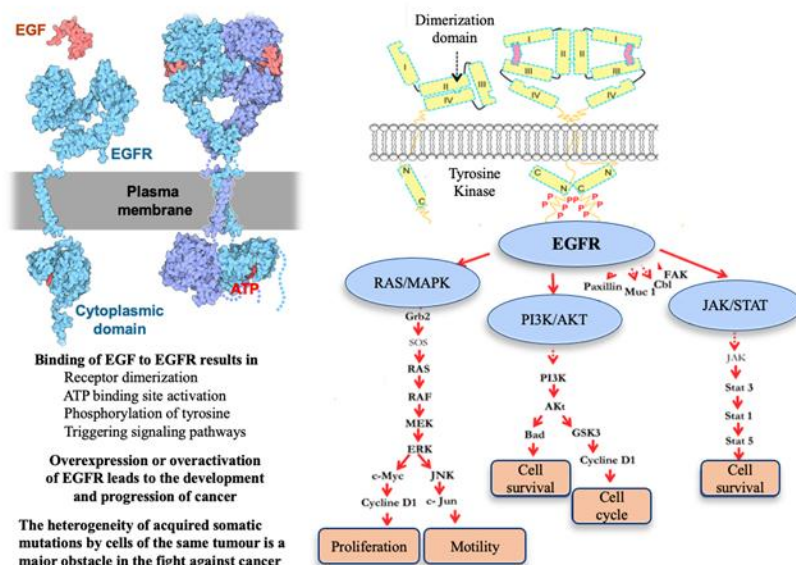
Fig. 1. Structure of monomeric and dimeric forms of EGFR and functions of the EGF ligand-bound receptor in cells.
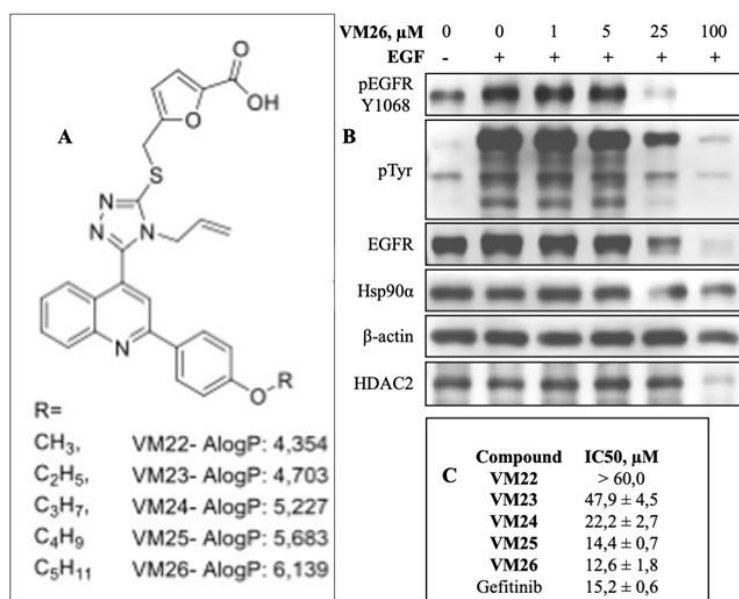

Fig. 2. Targeted protein degradation in cancer cells with polyfunctionalized heterocyclic compounds. **A** – furfuryl derivatives of 4-allyl-5-[2-(4'-alkoxyphenyl)quinolin-4-yl]-4H-1,2,4-triazole-3-thiol; **B** – compound **VM26** shows dose-dependent degradation of EGFR and other proteins and reduced phosphorylation mediated by EGFR in MDA MB68 cancer cells; **C** – the cytotoxicity of compounds (IC50) in MDA MB468 cancer cells grown with fetal bovine serum for 72 hours.
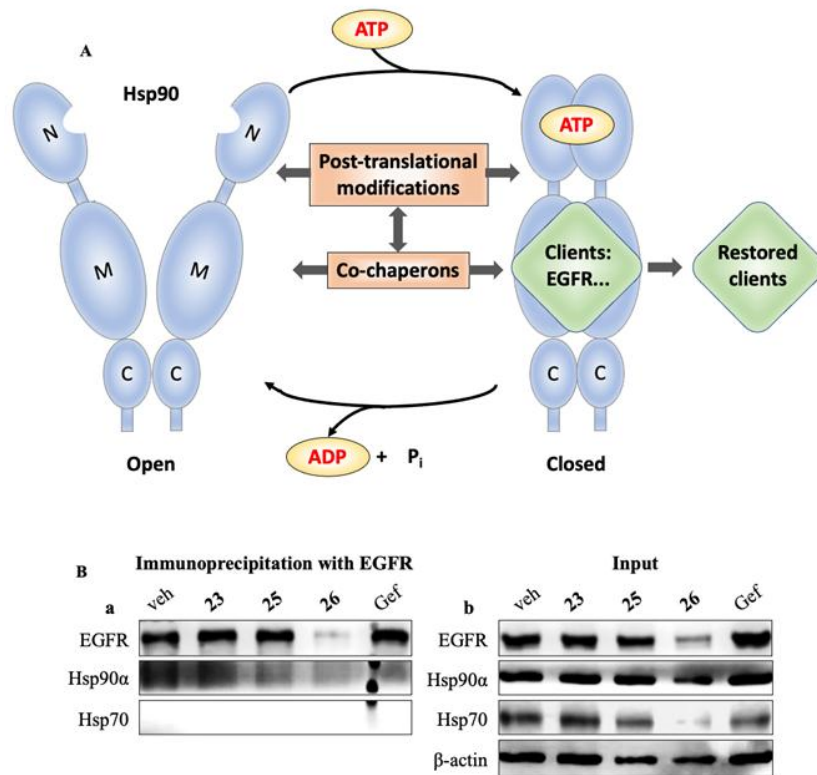
Fig. 3. Molecular structure and function domain of Hsp90α bound to EGFR. **A –** Activation of client proteins by the Hsp90α chaperone. Hsp90α in the open conformation dimerizes in the C-domain, and ATP binding leads to a conformation change to the closed form. Upon ATP hydrolysis, Hsp90α returns to the open conformation and is ready to start another chaperone cycle, regulated by cochaperone proteins. **B –** Hsp90α protein immunoprecipitated with anti-EGFR mAb (**a**), and input protein samples (**b**). Serum-starved MDA MB468 cells were treated with 25 μM **VM23**, **VM25**, **VM26**, or Gefetinib for 2 h. The proteins were collected on protein G conjugated to magnetic beads.
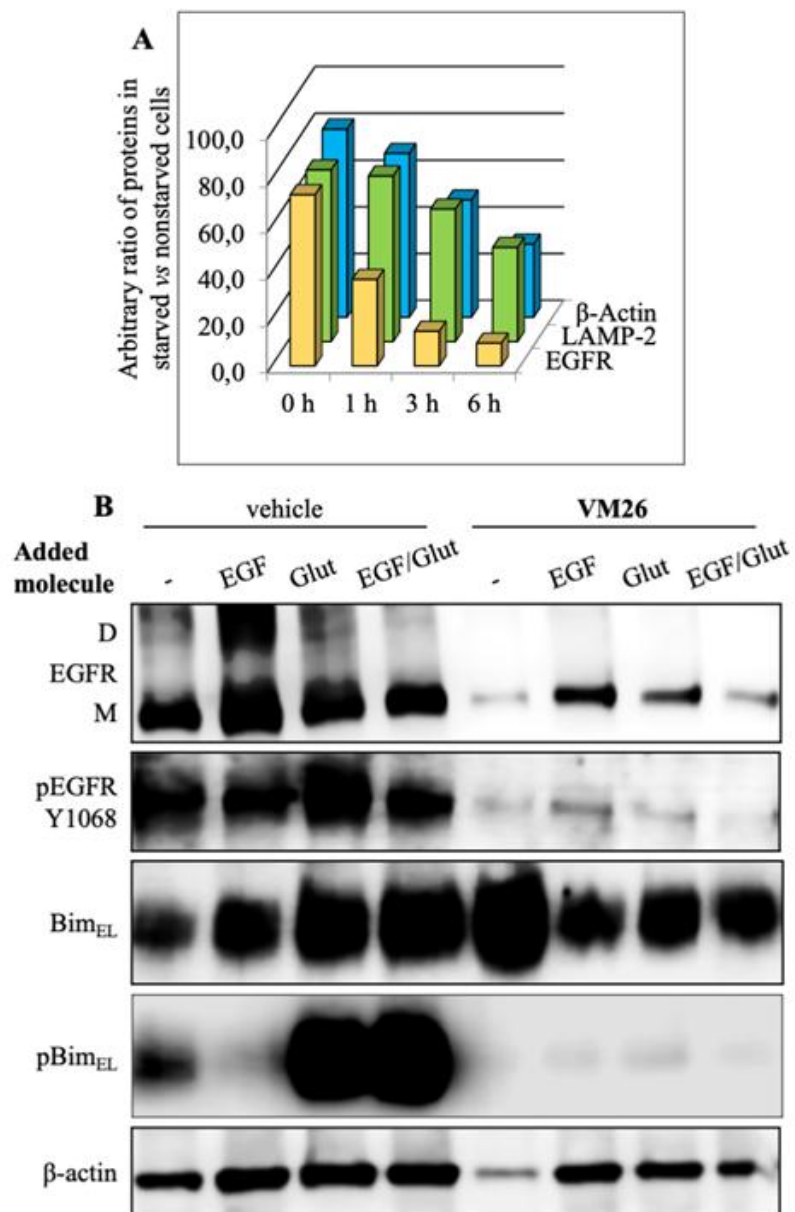
Fig. 4. Sequestration of Bim in MDA MB468 cancer cells by allosteric degraders of EGFR. **A** – Two step protein degradation in starved cells vs nonstarved cells considering 100% of each protein in nonstarved cells. Relative levels of proteins were estimated as their ratio. **B** – Impact of EGF and glutamine on protein expression and phosphorylation in untreated ells and treated with 25 µM **VM26** for 6 h in serum-deprived medium.
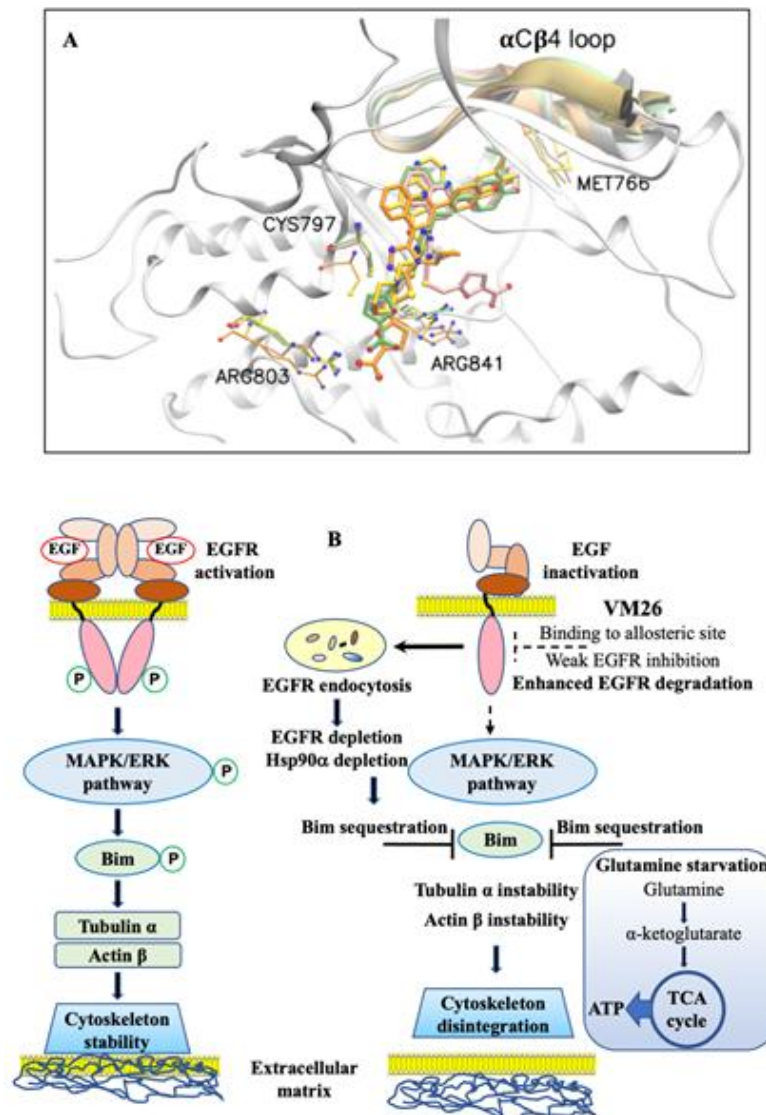
Fig. 5. Mechanism of action of EGFR-specific allosteric degraders on cancer cells. **A** – Structure of EGFR bound to **VM3** (orange), **VM25** (pink), and **VM26** (green) and Gefitinib (yellow). **B** – Compound **VM26** binds to a hydrophobic allosteric site in EGFR, inducing degradation of the receptor in endosomes. Depletion of EGFR, possibly with Hsp90α, leads to sequestration of Bim, followed by disintegration of the cytoskeleton and detachment of cancer cells from the extracellular matrix. Glutamine starvation causes a deficiency of α-ketoglutarate and an inability of cells to replenish the tricarboxylic acid (TCA) cycle and produce ATP. Double starvation of EGF and glutamine reinforces the cytoskeleton disintegration leading to cancer cell detachment-promoted death.

Endocytotic degradation of EGFR. Protein degradation in mammals depends on the initiation type of autophagy categorized as macro-autophagy, chaperone-mediated, and micro-autophagy [12]. Endocytosis of EGFR is a micro-autophagy process, which orchestrates cellular signaling networks, and can direct the fate of the receptor in cells. Ligand-bound EGFR undergoes endocytosis followed by recycling and/or degradation of the receptor by proteolytic enzymes in lysosomes fused to endosomes [13]. Low doses of EGF activate clathrin-dependent endocytosis, which promotes sustained EGFR signaling and is the main mechanism of EGFR endocytosis in tumors in vivo [14]. High doses of ligand additionally induce clathrin-independent endocytosis, which is the main lysosomal degradation pathway for reducing EGFR signaling [15]. Apparently, this process affects the fate of the receptor through ubiquitination at saturated concentrations [16].

We have synthesized furfuryl derivatives of 4-allyl-5-[2-(4'-alkoxyphenyl) quinolin-4-yl]-4H-1,2,4-triazole-3-thiol by combining various scaffolds in one molecule [7]. Alkyl ether substituents of different lengths were attached to the benzene ring to obtain new **VM** compounds that provide a differential increase in the sensitivity of the target protein to the action of proteases (Fig. 2A). The triple negative breast cancer cell line MDA MB468, in which the EGFR protein is overexpressed compared to a low-expressing ErbB2 counterpart, was used in our study. Experiments showed a significant suppression of EGFR tyrosine phosphorylation associated with a decrease in its expression due to protein degradation (Fig. 2B). The ability of the active compounds to simultaneously suppress tyrosine phosphorylation and to reduce EGFR levels and other functionally unrelated proteins suggested that the small molecules induce protein degradation by first targeting EGFR in cancer cells. Active **VM** compounds **VM25** and **VM26** demonstrated relatively high cytotoxicity comparable to gefitinib (Fig. 2C), a well-known first-generation anti-EGFR drug [17].

We assessed protein levels in serum-deprived cells in which EGFR expression was reduced by siRNA silencing [7]. Decreased levels of EGFR and Hsp90α were observed when cells were exposed to **VM** compounds compared to cells transfected with scrambled siRNA. We also evaluated the autophagy biomarkers LC3α/LC3β, considering that the accumulation of LC3β correlates with an increase in the number of autophagosomes, which degrade nutrient-starved proteins in cells [18]. Immunofluorescence microscopy was used to study autophagy biomarkers in the cells incubated with a lower concentration of compounds for a shorter time [7]. The LC3β protein emitted a strong fluorescent signal upon exposure to **VM26** relative to the vehicle, indicating the compound resulted in a rapid response of the autophagy mechanism.

Chaperone HSP90α promotes autophagic degradation of EGFR. Heat shock proteins (HSPs) play a crucial role in the process of protein folding during proliferation, invasion, metastasis, and death of cancer cells. Among these proteins, HSP90α, a highly conserved molecular chaperone, has over 700 protein substrates known as client proteins [19]. HSP90α is involved in a variety of cellular processes beyond protein folding, which include DNA repair,

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immune response development, and neurodegenerative diseases. Other co-chaperones interact with HSP90α and regulate ATPase-associated conformational changes in the HSP90α dimer that occur during processing of client proteins (Fig. 3A). Therefore, this chaperone plays a key role in correcting the misfolding of client proteins and protecting them from ubiquitination and degradation by the 26S proteasome [20].

EGFR is a client protein for Hsp90α that in cooperation with Hsp70 controls and edits the proper folding and maturation of nascent polypeptides via a super-chaperone complex in normal and cancer cells [21]. The chaperone Hsp70 initially recognizes a misfolded client protein and then translocates the bound protein to Hsp90α, which promotes the maturation of the client protein [22]. The αCβ4 loop in EGFR moderately conserved in client protein kinases is likely to be recognized by Hsp90α [23]. The chaperone Hsp90α is highly expressed in cancer cells, and diminution of the Hsp90 machinery activity leads to the degradation of misfolded client proteins by cellular proteasome.

We studied the effect of **VM** compounds on the interaction of EGFR with Hsp90α by immunoprecipitation. The analysis showed that the 90-kDa Hsp90α diffuse band could be immunoprecipitated with EGFR from cell extracts treated with the three compounds, with the lowest chaperone yield in samples corresponding to **VM26** treatment (Fig. 3Ba). No band corresponding to Hsp70 was detected in immunoprecipitated extracts, whereas a 70-kDa protein was detected in input samples and at lower levels in cells exposed to compounds **VM25** and especially **VM26** (Fig. 3Bb). This means that the final chaperone-target protein complex does not contain Hsp70, which dissociated after providing for EGFR translocation to Hsp90α. A lower amount of Hsp90α in samples precipitated with anti-EGFR antibody indicates simultaneous degradation of the chaperone in the EGFR- Hsp90α complex due to binding of the **VM** compound. Obviously, a decrease in EGFR folding should promote greater degradation of the receptor protein, which may lead to a decrease in the phosphorylation flux in downstream signaling pathways.

Detachment of cancer cells from the extracellular matrix. The cytoskeleton consists of actin polymers and microtubules formed by tubulin polymers, which in concert with other proteins allow integrins to attach to the extracellular matrix [24]. EGFR phosphorylation status in downstream signaling pathways determines the functional state of integrins, which are transmembrane receptors that mediate cell adhesion to the extracellular matrix. Crucially, EGFR governs the normal functioning of the cytoskeleton through the MAPK/ERK pathway by phosphorylation of the proapoptotic Bim, a sensor protein important for interaction with microtubules [25]. Interruption of this signaling pathway by blocking Bim phosphorylation leads to the sequestration of the cytoskeleton and the detachment of healthy cells. Nutrient starvation impairs the EGFR signaling cascade, leading to the detachment of cells and ultimately to a programmed death pathway known as anoikis. Compared with healthy cells, cancer cells possess a higher tolerance to anoikis, and this seems to be involved in the metastatic progression of inflammatory tumors.

We observed that incubation of cancer cells with **VM** compounds reduced levels of β-actin and α-tubulin, cytoskeletal proteins commonly used as loading controls in Western blotting [7]. In addition, the compounds caused detachment of cancer cells from the extracellular matrix, especially under conditions of cell starvation for EGF or glutamine. Only traces of EGFR were detected in attached cells, and the receptor protein was absent in detached cells after exposure to the compounds in serum-supplemented medium.

To determine whether the destabilization of the cytoskeletal machinery is related to the status of the Bim sensor protein, protein expression was assessed by immunofluorescence imaging and Western blotting. Kinetic analyzes showed a transient and significant increase in BimEL after one hour of exposure to **VM26** in a serum-deprived medium, followed by a decrease in this protein level after three hours of exposure. Notably, a high level of BimEL expression was associated with a decrease in the amount of EGFR after one hour. The amount of lysosomal protease LAMP-2 and cytoskeletal protein β-actin decreased later compared to EGFR. This two-speed decrease in the abundance of functionally unrelated proteins (Fig. 4A) seems to reflect two processes: early and rapid degradation of EGFR by endocytosis, followed by slower disintegration of the cytoskeleton due to Bim sequestration.

To elucidate which major nutritional factors are involved in Bim induced sequestration, protein profiles were compared in serum-deprived cultures after addition of EGF or glutamine or both for 6 hours. Glutamine and its mixture with EGF and, to a lesser extent, EGF increased Bim_{EL} expression compared to vehicle or EGF alone (Fig. 4B). Moreover, a significant increase in the rate of phosphorylation was detected in Ser69 Bim_{EL}, which is likely due to increased phosphorylation at Tyr1068 in EGFR. Replenishment of the medium with a fresh portion of glutamine improved the functional state of Bim in the absence of **VM** compounds.

Serum-deprived cells treated with **VM26** in the presence of EGF, glutamine, or both had elevated expression levels of EGFR, LAMP-2, β-actin, and cleaved caspase 3 compared to low protein expression in the presence of compound alone (Fig. 4B). BimEL expression essentially increased after exposure to **VM26**, and no noticeable modulation was detected after the addition of EGF, glutamine, or both in the starved culture. Notably, **VM26** strongly suppressed Ser69 phosphorylation in BimEL, apparently associated with reduced phosphorylation in EGFR, regardless of the addition of EGF, glutamine, or both to the growth medium.

Targeted degradation of the receptor protein is of particular interest in prostate cancer. The traditional treatment for this cancer, which eventually develops into a castration-resistant form with a poor prognosis for patients, is androgen deprivation. Standard methods of radiation therapy, chemotherapy, hormonal therapy, and surgery are not considered fully effective in the treatment of advanced and metastatic forms of cancer. We evaluated **VM** compounds in the DU-145 prostate cancer cell line, characterized by an unusual decrease in EGFR levels in response to EGF action in an autocrine loop,

presumably associated with endocytosis [26]. Depletion of EGFR by **VM26** for three hours resulted in a decrease in BimEL expression and Ser69 phosphorylation in attached cells, which was associated with a larger number of cells detached from the extracellular matrix [7]. After a 24-hour incubation, **VM26** resulted in the disappearance of EGFR and β-actin in attached cells, regardless of the addition of EGF or glutamine to the starvation medium.

These results confirmed that **VM** compound-induced EGFR depletion drastically destabilizes the cytoskeleton, leading to detachment of cancer cells from the extracellular matrix and, ultimately, death resembling apoptotic anoikis. Depletion of HSP90α, which depends on the amount of chaperon-bound EGFR, can additionally contribute to cell detachment by reducing the ability of the Hsp90/Hsp70 system to properly fold a huge number of nutrient-prone proteins. Thus, both misfolded client proteins and nutrient-prone proteins can become targets for proteases in the endolysosomes and cytoplasm of cells treated with polyfunctional heterocyclic compounds.

Why does glutamine increase the anticancer capacity of EGFR degraders? Our data demonstrate the ability of allosteric degraders of EGFR to influence metabolic and energy balance in glutamine-deprived cancer cells. How does glutamine deficiency enhance **VM26** ability to kill cancer cells?

Cancer cells grow rapidly and require more energy for protein synthesis than normal cells. Glutamine is converted to α-ketoglutarate, which merges with the tricarboxylic acid cycle to form large amounts of ATP. Moreover, EGFR requires ATP to activate the catalytic ATP-binding site and more than thirty amino acids, including seven tyrosine residues, even if the receptor undergoes endocytosis [27]. The addition of glutamine to the culture medium is necessary due to the instability of this amino acid at 37°C. The ATPase activity of the chaperone Hsp90α provides energy-dependent correction of many misfolded client proteins, protecting them from ubiquitination and 26S proteasome-promoted degradation [20]. As Hsp90α levels decrease with EGFR depletion, misfolded glutamine-prone proteins become available for degradation by proteases. Therefore, the dependence of EGFR activation and related processes on the content of glutamine in cells can be formulated as «no glutamine, no EGFR signaling».

New philosophy of cancer chemotherapy. Chemotherapeutic interruption of the flow of MAPK/ERK phosphorylation by allosteric TKIs is a promising tool for reducing the metastatic spread of cancer cells [28]. Small molecular glues and heterobifunctional molecules have also been developed as targeted protein degraders to treat cancer and other diseases. PROTAC targeted protein degradation is particularly attractive for modulating proteins that are difficult to target with TKIs [29]. Other types of molecular glues based on the ability of thalidomide to act as a degrader constitute another important class of drug-like agents for the treatment of diseases [30]. Scientific progress in recent years and clinical trials of PROTAC degraders and thalidomide analogs in clinical phases I and II suggest that targeted protein degradation will become a key therapeutic option in the fight against cancer in the coming decades.

We have developed an alternative targeted protein degradation approach based on 4-allyl-5-[2-(4'-alkoxyphenyl)quinolin-4-yl]-4H-1,2,4-triazole-3-thiol derivatives, which bind EGFR [7]. These small degraders are completely different from the PROTAC and thalidomide degraders and are likely to be advantageous in terms of their action on cancer cells. The polyfunctional heterocyclic compounds target and degrade EGFR, resulting in a cascade of death-promoting events that resemble cancer cell cytotoxic killers rather than cancer cell proliferation cytostatic blockers. We believe that such an action opens a very attractive and effective prospect of cancer treatment.

New compounds first bind to a hydrophobic allosteric pocket located in the immediate vicinity of the ATP binding site (Fig. 5A). Molecular dynamics simulation has revealed that the short chain CH_3CH_2 in new compounds is not bulky enough to fill the hydrophobic allosteric pocket, while the longer chain $CH_3(CH_2)_4$ almost completely occupies this site. An important role in the binding of compounds to EGFR seems to be played by their reorientation from Arg803 to Arg841, which is consistent with the participation of Arg841 in the dynamic changes preceding the sulfenylation of Cys797 [7, 31]. This probably leads to the interaction of longer alkyl ether chains of the compounds with Met766 in the αCβ4 loop located near the ATP binding site. This rearrangement may accelerate and/or enhance the endocytic degradation of EGFR (Fig. 5B). Induced depletion of EGFR and probably its associated Hsp90α chaperone leads to sequestration of Bim, which provokes disintegration of the cytoskeleton. Therefore, two different authentic pathways of protein degradation metabolism, endocytic and cytoplasmic degradations, promote cell detachment. This course of logically connected events reflects the functional interplay that precedes the death of cancer cells.

The impact on the degradation of EGFR by new generation of small molecules is a fundamentally different rational way to reduce the activity of tyrosine kinase. Targeting EGFR degradation has an advantage over EGFR inhibition because it promotes a more specific interruption of Bim phosphorylation leading to the death of cancer cells. Unlike cytostatic TKIs against EGFR, allosteric degraders of EGFR affect cell survival rather than growth and induce cancer cell death like cytotoxic molecules. This unexpected biological scenario is reminiscent of the return of «immortal» cancer cells to programmed cell death, anoikis. This means that allosteric inhibitors of EGFR are not «cancer cell killers», but rather molecules that restore the lost ability of cancer cells to die like normal cells after a certain number of generations. Notably, reduction in tumor size by allosteric inhibitors of EGFR has been confirmed *in vivo* in a mouse model of sarcoma [32].

The proposed mechanism of targeted protein degradation indicates that allosteric degraders of EGFR are attractive and promising agents for chemotherapy of human metastatic tumors. Shutting down phosphorylation pathways by potent TKIs in proliferating cancer cells creates selective conditions for the emergence of different mutants through alternative mechanisms, such as H_2O_2 release, in the branched EGFR interactome in the tumor

microenvironment. Conversely, protein degradation due to EGFR depletion results in cancer cell death, leaving fewer cells to proliferate, making it less likely to create conditions for new mutations to occur. Thus, this study opens the door to research aimed at attenuating metastatic progression and reducing drug resistance in malignant tumors associated with aberrant EGFR behavior in cancer cells.

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**Development of Targeted EGFR Degradation
for Cancer Treatment**

Chemotherapeutic interruption of signaling pathways at receptor tyrosine kinases is an important strategy for attenuating cancer progression. We have synthesized a new generation of polyfunctionalized heterocyclic compounds that bind to an allosteric site in the tyrosine kinase domain of the epidermal growth factor receptor (EGFR). The bound compounds induce degradation of the receptor by endocytosis in cancer cells. The Hsp90α chaperone binds to EGFR and is significantly lost during endocytosis, thereby contributing to the reduction of client proteins. Induced EGFR depletion leads to inactivation of downstream signaling due to sequestration of the Bim sensor protein for cytoskeletal proteins, provoking cell detachment from the extracellular matrix and ultimately cancer cell death. The role of glutamine in maintaining the phosphorylation status of EGFR-mediated signaling pathways can be postulated as «no glutamine, no EGFR signaling». Targeted degradation of EGFR is attractive for aiming to attenuate metastatic progression and to override the drug resistance of malignant tumors.

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**Քաղցկեղի բուժման նպատակային EGFR-ի
քայքայման մշակում**

Թիրոզինկինազային ընկալիչների ազդանշանային ուղիների արգելափակումը քաղցկեղի առաջընթացը դանդաղեցնելու հնարավոր ռազմավարություն է: Մենք սինթեզել ենք բազմաֆունկցիոնալ հետերոցիկլիկ միացությունների նոր սերունդ, որոնք կապվում են էպիդերմիսի աճի գործոնի ընկալիչի (EGFR) թիրոզինկինազային տիրույթի ալոստերիկ տեղամասին: Միացությունները էնդոցիտոզով քաղցկեղի բջիջ-ներում առաջացնում են ընկալիչների դեգրադացիա: EGFR-ի հետ կապված Hsp90α շապերոնը զգալիորեն կորչում է էնդոցիտոզի ժամանակ՝ դրանով իսկ նպաստելով

սպիտակուցների քայքայմանը: EGFR-ի քայքայումը հանգեցնում է ազդանշանի ապա-ակտիվացման՝ ցիտոկմախքի համար Bim սենսորային սպիտակուցի սեկվեստրա-ցիայի պատճառով, ինչը հանգեցնում է բջիջների բաժանման արտաբջջային մատ-րիցից և քաղցկեղի բջիջների մահվան: Գլուտամինի դերը EGFR-ով միջնորդավորված ազդանշանային ուղիների ֆոսֆորիլացման կարգավիճակի պահպանման գործում կարելի է նկարագրել որպես «ոչ գլուտամին, ոչ ազդանշան EGFR-ի համար»: EGFR-ի նպատակային դեգրադացիան գրավիչ է մետաստատիկ առաջընթացը թուլացնելու և չարորակ ուռուցքներում դեղորայքային դիմադրողականությունը ճնշելու համար:

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Разработка направленной деградации EGFR
для лечения рака

Химиотерапевтическое прерывание сигнальных путей на рецепторах тиро-зинкиназ является важной стратегией для ослабления прогрессирования рака. Синтезировано новое поколение полифункциональных гетероциклических соеди-нений, которые связываются с аллостерическим сайтом в тирозинкиназном домене рецептора эпидермального фактора роста (EGFR). Связанные соединения вызывают деградацию рецептора путем эндоцитоза в раковых клетках. Шаперон Hsp90α, связываясь с EGFR, значительно теряется во время эндоцитоза, тем самым способствуя деградации белков-клиентов. Индуцированное истощение EGFR приводит к инактивации передачи сигналов из-за секвестрации сенсорного белка Bim для цитоскелета, провоцируя отделение клеток от внеклеточного матрикса и в итоге гибель раковых клеток. Роль глутамина в поддержании статуса фосфорилирования сигнальных путей, опосредованных EGFR, можно постулировать как «нет глутамина, нет передачи сигналов через EGFR». Направленная деградация EGFR может быть применена для ослабления метастатического прогрессирования и подавления лекарственной устойчивости злокачественных опухолей.

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