The CUL4A ubiquitin ligase is a potential therapeutic target in skin cancer and other malignancies

Jeffrey Hannah¹ and Peng-Bo Zhou¹,²

Abstract
Cullin 4A (CUL4A) is an E3 ubiquitin ligase that directly affects DNA repair and cell cycle progression by targeting substrates including damage-specific DNA-binding protein 2 (DDB2), xeroderma pigmentosum complementation group C (XPC), chromatin licensing and DNA replication factor 1 (Cdt1), and p21. Recent work from our laboratory has shown that Cul4a-deficient mice have greatly reduced rates of ultraviolet-induced skin carcinomas. On a cellular level, Cul4a-deficient cells have great capacity for DNA repair and demonstrate a slow rate of proliferation due primarily to increased expression of DDB2 and p21, respectively. This suggests that CUL4A promotes tumorigenesis (as well as accumulation of skin damage and subsequent premature aging) by limiting DNA repair activity and expediting S phase entry. In addition, CUL4A has been found to be up-regulated via gene amplification or overexpression in breast cancers, hepatocellular carcinomas, squamous cell carcinomas, adrenocortical carcinomas, childhood medulloblastomas, and malignant pleural mesotheliomas. Because of its oncogenic activity in skin cancer and up-regulation in other malignancies, CUL4A has arisen as a potential candidate for targeted therapeutic approaches. In this review, we outline the established functions of CUL4A and discuss the E3 ligase’s emergence as a potential driver of tumorigenesis.

Key words Ubiquitination, cullins, DNA damage, cell cycle regulation, skin cancer, therapeutic targets

For most cellular proteins, stability and degradation are regulated by the ubiquitin-proteosome system in which proteins are posttranslationally modified with polyubiquitin chains and recruited to the 26S proteasome for proteolytic degradation. Although ubiquitin molecules are activated by E1 enzymes and physically conjugated to substrate proteins by E2 enzymes, E3 ubiquitin ligases are responsible for conferring substrate specificity and facilitating the interaction between substrates and E2 enzymes. The members of the largest family of E3 ligases in eukaryotes are cullin-RING ubiquitin ligases (CRLs), which share a modular configuration: RING box 1 (Rbx1) and E2 recruitment at the C-terminus of the cullin scaffold, and one or multiple substrate-recruiting proteins at the N-terminus. For instance, the N-terminus of cullin 4A (CUL4A) directly binds the adaptor protein damage-specific DNA-binding protein 1 (DDB1), which then recruits a number of possible substrate receptors known as DDB1-CUL4–associated factors (DCAFs) to assemble the cullin-RING ligase 4A (CRL4A) complex. These DCAFs directly bind to target proteins, enabling CRL4A to regulate their stability and activity and influence a wide variety of key processes of the cell—often, in a multi-tiered manner. For example, when cells experience ultraviolet (UV) irradiation and DNA damage, CRL4A becomes activated and targets multiple regulators of the cell cycle and DNA replication pathways as well as DNA repair proteins. It is noteworthy that CUL4A has a highly similar family member known as CUL4B that is also able to bind DDB1 and exhibits functional redundancy with CUL4A, as well as distinct activities. However, within the CUL4 subfamily, CUL4A is thought to be the major regulator of the processes and substrates described in this review.

Although DDB1 is now known to primarily function as a substrate adaptor to recruit DCAFs and, by extension, substrates to CUL4A and the ubiquitin-conjugating machinery, it was first identified as a
damaged DNA-binding (DBB) protein that exists in a heterodimeric complex with DDB2\(^{[15,16]}\). DDB2 is the damage recognition subunit that initiates the nucleotide excision repair (NER) pathway. DDB2 is also known as xeroderma pigmentosum (XP) complementation group E (XPE). XP is a condition that is caused by mutations in one of the genes that code for components of the NER pathway (except for the variant form of XP, which is due to loss of a polymerase involved in lesion bypass during DNA replication). As a result, these patients are hypersensitive to UV rays and sunlight and develop frequent squamous cell carcinomas and other skin malignancies. When cells undergo UV irradiation, DDB2 preferentially and directly binds DNA lesions such as 6-4 photoproducts and cyclopyrimidine dimers and recruits other components of the NER machinery to the damaged DNA\(^{[17]}\). Shortly after this, DDB2 is ubiquitinated and targeted for degradation by CRL4A, which is also active under these conditions\(^{[15,18-20]}\). In addition to DDB2, another well-characterized NER protein and damaged DNA sensor, XPC, has also been shown to be ubiquitinated by CRL4A\(^{[12,20]}\). Interestingly, both human DDB2 and XPC are transcriptional targets of the p53 tumor suppressor protein, further demonstrating that CUL4A acts in opposition to the DNA damage response\(^{[18,21]}\).

In addition to DNA repair, CRL4A also regulates the cell cycle by targeting the cyclin-dependent kinase inhibitor p21, which delays S phase entry by inhibiting the Cyclin E/Cdk2 complex, and SET domain-containing protein 7 (Set7) which is a histone H4 Lys20 monomethyltransferase (H4K20me1) that regulates cell cycle progression in G2/M phase\(^{[10,11,22-24]}\). CRL4A also targets Cdt1, which is a DNA replication licensing factor that ensures that the origins initiate replication only once per cell cycle\(^{[7,6,27]}\). Interestingly, for all of these targets, CRL4A uses the DCAF Cdt2, which binds substrates in a proliferating cell nuclear antigen (PCNA)–dependent manner. That is, all of the substrates contain the PCNA-binding motif, known as the PIP box, which is required for recognition by the CRL4A ubiquitin ligase. This dependence on PCNA and the observed delays in S phase entry in Cull4a-deficient cells demonstrates that CUL4A, which serves as the basis of the CRL4A complex, is an important regulator of the cell cycle. Upon UV irradiation and subsequent DNA damage, CRL4A becomes activated and targets these regulators of the cell cycle and replication (along with the DNA repair proteins described above). Therefore, it appears that CUL4A promotes progression of the cell cycle under normal conditions as well as when the cell experiences genotoxic stress. In the latter case, CUL4A arguably antagonizes cellular DNA damage response by degrading crucial NER (e.g., DDB2 and XPC) and checkpoint proteins (e.g., p21), thus reducing repair activity and shortening the time cells have to address genetic insults before replication is initiated\(^{[12,19]}\).

Figure 1. The cullin-RING ligase 4A (CRL4A) ubiquitin ligase complex. Cullin 4A (CUL4A) serves as the scaffold that uses two adaptors—RING domain-containing Rbx1 and triple WD40 domain-containing damage-specific DNA-binding protein 1 (DDB1)—to recruit the ubiquitin-charged E2 ubiquitin-conjugating enzyme and the DDB1-CUL4A-associated factors (DCAF) substrate receptor that is loaded with the substrate, respectively. This assembly of individual subunits of the complex places the E2 and the substrate in close proximity, facilitating transfer of the ubiquitin molecule from the enzyme to the substrate protein. The three WD40 β-propeller (SP) domains of DDB1 are designated BPA, BPB, and BPC. S, substrate; Ub, ubiquitin; E2, ubiquitin-conjugating enzyme.
initiation of DNA synthesis and, thereby, conferring resistance to UV-induced skin tumors in mice.

The data from this report strongly implicate CUL4A as an influential factor in UV-induced skin cancer. CUL4A seemingly promotes the accumulation of DNA damage by reducing the threshold levels of DNA repair proteins as well as promoting cell cycle progression. Most interestingly, our work has revealed that human cells are evidently functioning below peak levels of DNA repair capacity (Figure 2). That is, stabilization of the DDB2 and XPC repair proteins as well as the checkpoint protein p21 allowed Cul4a-deficient cells to resolve UV-induced DNA damage more efficiently than their Cul4a-proficient counterparts. As such, targeted inhibition of the CRL4A ubiquitin ligase is expected to provide new avenues for cancer prevention. Moreover, UV rays expedite the skin aging process as a result of increased accumulation of unreppaired DNA lesions, an unfortunate side effect of certain professions or lifestyles that involve routine, prolonged exposure to sunlight. In principle, targeted inhibition of the CRL4A ubiquitin ligase complex may reduce skin damage and slow down the aging effect caused by chronic exposure to harmful UV rays. Conversely, excess CUL4A is expected to cause premature destruction of rate-limiting NER and checkpoint factors, resulting in increased accumulation of unrepaired DNA lesions and genomic instability. Evidence from genomic analyses conducted by other groups reveal an up-regulation of CUL4A in breast cancer\cite{28,29}, hepatocellular carcinomas\cite{30}, squamous cell carcinomas\cite{31}, adrenocortical carcinomas\cite{32}, childhood medulloblastomas\cite{33}, and malignant pleural mesotheliomas\cite{34} as a result of gene amplification or overexpression.

The role of CUL4A in promoting tumorigenesis is anticipated to be the focus of future investigations. Indeed, our work suggests the intriguing possibility that a CRL4A inhibitor could be used to prevent UV-associated pathologies such as skin cancer and premature aging. Furthermore, we believe that targeting Cul4a presents low risk to patient health because, in our studies, Cul4a-deficient mice did not show any adverse phenotypes (which could potentially be explained by CUL4B compensating for the loss of CUL4A). In summary, CUL4A directly regulates processes that are relevant to tumorigenesis, and is more highly expressed in a number of cancers; loss of CUL4A expression is predicted to have minimal effects in humans. For all these reasons, CUL4A is an enticing potential therapeutic target in treating cancer and premature aging.

![Figure 2. Proposed Inhibitory role of CRL4A ubiquitin ligase in controlling the threshold of DNA repair and DNA damage checkpoint pathways after ultraviolet (UV) irradiation. The DNA damage response in mammals is normally operating below optimal capacity due to targeted ubiquitination and degradation of nucleotide excision repair (NER) sensors (damage-specific DNA-binding protein 2 and xeroderma pigmentosum complementation group C) and G1/S checkpoint effector (p21) by CRL4A. Cells with high levels of CUL4A expression are further compromised in their NER and checkpoint capacity and, as a result, are expected to accumulate unrepaired DNA lesions, leading to genomic instability and subsequent predisposition to skin cancer and aging. Conversely, inhibition of CUL4A promotes both NER and checkpoint pathways, thereby conferring better protection against UV- or chemical carcinogen–induced tumorigenesis.](image-url)
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