CYLD and Skin Squamous Tumors

Subjects: Oncology

Contributor: M. Llanos Casanova

Cylindromatosis (CYLD) is a deubiquitinase (DUB) enzyme that was initially characterized as a tumor suppressor of adnexal skin tumors in patients with CYLD syndrome. Later, it was also shown that the expression of functionally inactive mutated forms of CYLD promoted tumor development and progression of non-melanoma skin cancer (NMSC). And, recently it has been demonstrated that a moderate increase in CYLD wildt ype levels inhibits in vivo in the development of skin squamous cell tumors.

Keywords: CYLD ; NF-κB ; epidermal differentiation ; non-melanoma skin cancer ; skin tumor suppressor ; apoptosis ; angiogenesis ; inflammation ; skin squamous cell tumor inhibition

1. Introduction

CYLD is a tumor-suppressor gene that encodes a K63 deubiquitinase enzyme. It directly regulates multiple key signaling cascades, such as the NF-κB, JNK, and the MAPK pathways, and it is a crucial regulator of diverse cellular processes such as immune responses, inflammation, death, and proliferation [1]. It was shown that genetic alterations of CYLD—rendering a catalytically inactive protein—led to the formation of tumors in patients with CYLD cutaneous syndrome [2][3][4]. Later, genome sequencing approaches have revealed somatic *CYLD* alterations in other numerous human cancers, including non-small cell lung cancer, melanoma, glioblastoma, breast, ovarian, bladder, colon, and head and neck cancer [5]. In addition, the correlation of CYLD downregulation with tumor development and progression of many types of cancers such as hepatocarcinoma, colon, and breast tumors [6][7][8] has also been described.

Among the diverse human neoplasias, NMSC are the most common malignancies. The skin consists of three layers: epidermis, dermis, and hypodermis, and it contains specialized structures, such as hair follicles (HF) and sebaceous and sweat glands. The epidermis is a stratified epithelium composed mainly of keratinocytes; among them, those of the basal layer are proliferative, move to the suprabasal layers, and gradually differentiate, giving rise to the outermost squamous cornified cell layer that is shed [9]. A balance between keratinocyte proliferation and differentiation is mandatory to maintain epidermal homeostasis; otherwise, numerous pathologies arise, including NMSC. Although dysregulation of the *CYLD* gene was established to be responsible for the development of skin appendage-derived tumors in CYLD syndrome patients [2][3][4], posteriorly, it was reported that the catalytic inactivation of CYLD was involved in the development and progression of skin squamous cell carcinomas (SCCs) [10][11] [12][13].

Basal cell carcinomas (BCCs) and SCCs represent the vast majority of the NMSC diagnosed [14] and 90% of skin cancers in the Caucasian population; of these, SCC can be very aggressive and almost 5% metastasize; therefore, due to its high incidence, the mortality caused by aggressive skin SCCs is reaching relevant numbers [15]. This and the fact that the incidence of NMSC has been increasing at an alarming rate in recent years, even in the population under 40 years of age, make NMSC an important health problem [16].

NF-κB is a ubiquitous and evolutionarily conserved transcription factor that regulates the expression of genes involved in the transformation, survival, proliferation, invasion, angiogenesis, and metastasis of tumor cells. It is composed of dimers of five members, of which p65/p50 is the predominant dimer in the skin [17]. In resting cells, NF-κB is maintained inactive while it has been found to be constitutively active most type of cancers including skin SCCs [18][19]: NF-κB inhibition in keratinocytes prevents tumor development by acting both during the initiation and promotion phases of skin carcinogenesis [20].

Results obtained in immunocompetent transgenic mice show that a moderate increase in the wild type CYLD expression levels prevents the development and progression of skin squamous cell tumors, mainly through the attenuation of NF-κB activation, reduction of tumor proliferation, increased tumor cell differentiation, and inhibition of both tumor inflammation and angiogenesis [21]. These results have important clinical significance and make *CYLD* appear as a promising therapeutic target of skin SCCs.

2. CYLD inhibits the development of skin carcinomas in immunocompetent mice

Transgenic mice expressing the wild type form of CYLD under the control of the K5 promoter (K5-CYLDwt mice) were generated (Figure 1, https://doi.org/10.3390/ijms22136736). Properties of their skin were analyzed by WB and immunohistochemistry (Figure 2, https://doi.org/10.3390/ijms22136736), and studied the survival and proliferating characteristics of primary keratinocytes (Figures 3 ,4, https://doi.org/10.3390/ijms22136736), as well as their predisposition to develop skin SCCs when subjetced to chemical skin carcinogenesis (Figures 5-8, https://doi.org/10.3390/ijms22136736). As a result, it was found a reduced activation of NF-κB in the skin of K5-CYLDwt mice in response to TNF-α; accordingly, when subjected to insults, K5-CYLDwt keratinocytes were prone to apoptosis and were protected from an excessive hyperproliferation. Skin carcinogenesis assays showed the inhibition of tumor development in K5-CYLDwt mice.

3. Conclusions

Studies of skin carcinogenesis in nude mice showed that the lack of the catalytic function of CYLD increased the aggressiveness of cutaneous SCCs [10] [22], i.e., the expression of the mutant $CYLD^{C/S}$, which acts as a dominant negative inhibiting the deubiquitination function of the endogenous CYLD, enhanced tumor cell proliferation, survival and tumor angiogenesis (this last considered a prominent feature of skin tumor progression) both in mouse and human skin SCCs [10][22][23]. Additionally, it was showed that overexpression of wild type CYLD in human skin SCC cells led, in xenograft assays in nude mice, to the development of more differentiated tumors with less efficient angiogenesis [22]. Later, it was shown that a modest increment in the expression of CYLD in K5-CYLDwt mice did not have any deleterious effect on normal keratinocytes, although it confered them a greater capacity to differentiate, and attenuated their responses to environmental stresses on the skin (such as TNF-α + Chx or TPA application), mainly through an increment in cell apoptosis and reduction in cell proliferation. On the contrary, a moderate increase in CYLD levels exerted a relevant role counteracting the damaging effects of transformed keratinocytes, since it prevented in a great manner the development of skin carcinomas, and those scarce tumors arisen exhibited signals of better prognosis, such as lower size, decreased proliferation, more differentiated phenotype, lower number of inflammatory cells and a network of small and mature blood vessels.

Altogether, these results suggest that wild type CYLD is able to repress NMSC development and progression *in vivo*, in immunocompetent transgenic mice, offering a powerful anti-cancer target against skin SCCs.

Inhibition of NF-kB activation in keratinocytes and skin tumors of mice with moderately increased CYLD expression.

Data suggesting the role of CYLD as a tumor suppressor of skin SCCs *in vivo* are in agreement with those of *Cyld*-/- mice, indicating that mice lacking the *Cyld* gene exhibited increased susceptibility to the development of skin cancer upon chemical carcinogenesis experiments [24]; they also agree with results showing that transgenic mice lacking the deubiquitinase function of CYLD in keratinocytes spontaneously develop skin tumors [11].

Attenuation of the activation of NF-κB in keratinocytes emerges as a likely mechanism through which CYLD exerts its function as a tumor suppressor of skin tumors, since diminished activation of NF-kB was observed in the scarce tumors developed by CYLD-overexpressing keratinocytes; in turn, NF-kB inhibition may inhibits responses such as inflammation, cell proliferation, and angiogenesis (largely responsible for tumor development and progression [25] cintributing to the better prognosis of tumors with moderately increased CYLD expression (https://doi.org/10.3390/ijms22136736). In fact, many different human cancers, both hematological malignancies and solid tumors, have been linked to constitutive NF-kB activation, among them skin SCCs [18].

Tumors with a modest increase in CYLD expression show a more differentiated and less proliferative phenotype, and diminished tumor inflammation and angiogenesis.

Tumors with higher expression of CYLD wild type are more differentiated and have almost no expression of K13, a keratin indicative of malignant progression of NMSC [10]. These results are in agreement with the role of CYLD as a positive regulator of epidermal differentiation previously described in human HaCaT keratinocytes as well as in mouse and human tumor epidermal cells [22]. Additionally, tumors overexpressing CYLD wild type were smaller, which could be due to their

lower proliferation as well as to the minor development of a network of blood vessels that would impair the nutrition of tumor cells. The role of CYLD as an inhibitor of keratinocyte proliferation was previously reported in xenografts assays and in *cyld*-deficient mice [10][26].

A relevant feature of tumors showing a moderate increase in CYLD expression levels is its lower inflammation and angiogenesis. The activation of NF-κB is an established pathway in the mediation of inflammation, which in turn is a major mediator of tumor development and progression of mouse and human skin SCCs [27][28]. Among other effects, inflammatory cells promote angiogenesis, favoring the development of the vascular network in tumors [29]. Angiogenesis is a relevant process which plays a key role in tumor progression, since a robust network of blood vessels makes possible the supply of oxygen and nutrients needed for cancer progression [23] [30]. Therefore, the lower number and better prognosis of tumors with higher levels of CYLD wild type might be the consequence of the decreased inflammatory environment of the tumor cells as well as to the reduced dimensions and mature nature of blood vessels. In turn, the reduced size of the blood vessels could contribute to the more differentiated phenotype of tumors with higher amounts of CYLD wild type, as the enlargement of angiogenic blood vessels is the main vasculature change that allows tumor progression [23]. Data showing the anti-angiogenic role of CYLD in skin tumors developed in immunocompetent mice are in agreement with those obtained in studies of xenograft carcinogenesis in nude mice, showing that CYLDwt overexpression in SCCs reduced the diameter of the tumor blood vessels and augmented its maturity [22]. They are also consistent with data showing that the impaired catalytic function of CYLD in tumor epidermal cells enhanced angiogenesis in both mouse and human skin SCCs, contributing to its malignant progression and metastatic behavior [10][22][31]. Additionally, it has been reported that the main mechanisms that led to the development of spontaneous skin tumors in mice lacking the deubiquitinase function of CYLD is the chronic activation of NF-κB and the constitutive inflammation in the skin of these mice [11]. Taken together, the avalibale data suggest that a moderate increase in CYLD levels in keratinocytes is able to inhibit inflammation and angiogenesis in skin SCCs and then act as a tumor suppressor of NMSC in mice with an undamaged immune system.

Relevance of CYLD wild type expression for clinical management of skin SCCs.

The results commented above suggest that a moderate increase in levels of CYLD might be useful for anti-skin cancer therapy. Until now, evidence of regulation of CYLD expression by pharmacological agents is scarce in the literature, but it has been reported that it may be possible to indirectly regulate CYLD levels by agents that act on regulatory factors for CYLD, such as Serum Response Factor (SRF) or kinase inhibitors [1]; also, levels of CYLD may be indirectly increased by allosteric inhibition of Caspase 8 (which cleaves CYLD) by the pan-caspase inhibitor zVAD-FMK [32]. Caspase-8 is upregulated and localized to the nucleus in multiple human cancers, correlating with resistance to therapy and poor clinical outcome: it promotes NF-κB-dependent expression of several cytokines, angiogenesis, and tumorigenesis [33], suggesting that inhibition of Caspase-8 might be an interesting promising regulator of CYLD levels in cancer. In addition, the role of p38 as a mediator of the positive regulation of CYLD expression by SRF has been described [34], and suggest that the use of p38-inhibitors could be an appropriate approximation to favor CYLD-mediated inhibition of skin squamous cell tumor development [35].

It is now clear that cancerous phenotypes result from the dysregulation of more than 500 genes at multiple steps in cell signaling pathways [34][36]. This indicates that inhibition of a single gene product or cell signaling pathway is unlikely to prevent or treat cancer. However, most current anticancer therapies are based on the modulation of a single target. Therefore, results showing that the modulation of a single gene, *CYLD*, is able to regulate multiple targets in skin tumors are very relevant from a clinic point of view, and would provide a huge advantage as a cancer treatment, since CYLD controls many of the major features dysregulated in cancer, such as cell proliferation, differentiation, cell survival, angiogenesis and inflammation.

References

1. Bryan Mathis; Yimu Lai; Chen Qu; Joseph S. Janicki; Taixing Cui; CYLD-mediated signaling and diseases.. *Current Drug Targets* **2015**, *16*, 284-94, 10.2174/1389450115666141024152421.
2. Brummelkamp, T.R.; Nijman, S.M.; Dirac, A.M.; Bernards, R. Loss of the cylindromatosis tumour suppressor inhibits apoptosis by activating NF-kappaB. Nature 2003, 424, 797–801.
3. Kovalenko, A.; Chable-Bessia, C.; Cantarella, G.; Israel, A.; Wallach, D.; Courtois, G. The tumour suppressor CYLD negatively regulates NF-kappaB signalling by deubiquitination. Nature 2003, 424, 801–805.

4. Trompouki, E.; Hatzivassiliou, E.; Tsichritzis, T.; Farmer, H.; Ashworth, A.; Mosialos, G. CYLD is a deubiquitinating enzyme that negatively regulates NF-kappaB activation by TNFR family members. Nature 2003, 424, 793–796.
5. The AACR Project GENIE Consortium; AACR Project GENIE: Powering Precision Medicine through an International Consortium. *Cancer Discovery* **2017**, *7*, 818-831, 10.1158/2159-8290.cd-17-0151.
6. Hellerbrand, C.; Bumes, E.; Bataille, F.; Dietmaier, W.; Massoumi, R.; Bosserhoff, A.K. Reduced expression of CYLD in human colon and hepatocellular carcinomas. Carcinogenesis 2007, 28, 21–27.
7. Kinoshita, H.; Okabe, H.; Beppu, T.; Chikamoto, A.; Hayashi, H.; Imai, K.; Mima, K.; Nakagawa, S.; Yokoyama, N.; Ishiko, T.; et al. CYLD downregulation is correlated with tumor development in patients with hepatocellular carcinoma Down-regulation of the Tumor Suppressor CYLD Enhances the Transformed Phenotype of Human Breast Cancer Cells. Mol. Clin. Oncol. 2013, 1, 309–314.
8. Orfanidou, T.; Xanthopoulos, K.; Dafou, D.; Pseftogas, A.; Hadweh, P.; Psyllaki, C.; Hatzivassiliou, E.; Mosialos, G. Down-regulation of the Tumor Suppressor CYLD Enhances the Transformed Phenotype of Human Breast Cancer Cells. Anticancer Res. 2017, 37, 3493–3503.
9. Fuchs, E. Epidermal differentiation: The bare essentials. J. Cell Biol. 1990, 111, 2807–2814.
10. J P Alameda; R Moreno-Maldonado; Manuel Navarro; A Bravo; Angel Ramirez; Angustias Page; J L Jorcano; M J Fernández-Aceñero; M. Llanos Casanova; An inactivating CYLD mutation promotes skin tumor progression by conferring enhanced proliferative, survival and angiogenic properties to epidermal cancer cells. *Oncogene* **2010**, *29*, 6522-6532, 10.1038/onc.2010.378.
11. Josefa P. Alameda; Angel Ramirez; Rosa A. García-Fernández; Manuel Navarro; Angustias Page; José C. Segovia; Rebeca Sanchez; Cristian Suárez; Jesus M Paramio; Ana Bravo; et al. Premature aging and cancer development in transgenic mice lacking functional CYLD. *Aging* **2019**, *11*, 127-159, 10.18632/aging.101732.
12. de Marval, P.M.; Lutfeali, S.; Jin, J.Y.; Leshin, B.; Selim, M.A.; Zhang, J.Y. CYLD Inhibits Tumorigenesis and Metastasis by Blocking JNK/AP1 Signaling at Multiple Levels. Cancer Prev. Res. 2011, 4, 851–859.
13. Masoumi, K.C.; Shaw-Hallgren, G.; Massoumi, R. Tumor Suppressor Function of CYLD in Nonmelanoma Skin Cancer. J. Skin Cancer 2011, 2011, 614097.
14. Limmer, B.L. Nonmelanoma skin cancer: Today's epidemic. Tex. Med. 2001, 97, 56–58.
15. Karia, P.S.; Han, J.; Schmults, C.D. Cutaneous squamous cell carcinoma: Estimated incidence of disease, nodal metastasis, and deaths from disease in the United States, 2012. J. Am. Acad. Dermatol. 2013, 68, 957–966.
16. Christenson, L.J.; Borrowman, T.A.; Vachon, C.M.; Tollefson, M.M.; Otley, C.C.; Weaver, A.L.; Roenigk, R.K. Incidence of basal cell and squamous cell carcinomas in a population younger than 40 years. JAMA 2005, 294, 681–690.
17. Ghosh, S.; Karin, M. Missing pieces in the NF-kappaB puzzle. Cell 2002, 109, S81–S96.
18. Véronique Baud; Michael Karin; Is NF-κB a good target for cancer therapy? Hopes and pitfalls. *Nature Reviews Drug Discovery* **2009**, *8*, 33-40, 10.1038/nrd2781.
19. Budunova, I.V.; Perez, P.; Vaden, V.R.; Spiegelman, V.S.; Slaga, T.J.; Jorcano, J.L. Increased expression of p50-NF-kappaB and constitutive activation of NF-kappaB transcription factors during mouse skin carcinogenesis. Oncogene 1999, 18, 7423–7431.
20. Kim, C.; Pasparakis, M. Epidermal p65/NF-kappaB signalling is essential for skin carcinogenesis. EMBO Mol. Med. 2014, 6, 970–983.
21. Josefa Alameda; Verónica García-García; Silvia López; Ana Hernando; Angustias Page; Manuel Navarro; Rodolfo Moreno-Maldonado; Jesús Paramio; Ángel Ramírez; Rosa García-Fernández; et al. CYLD Inhibits the Development of Skin Squamous Cell Tumors in Immunocompetent Mice. *International Journal of Molecular Sciences* **2021**, *22*, 6736, 10.3390/ijms22136736.
22. J P Alameda; M J Fernández-Aceñero; R Moreno-Maldonado; Manuel Navarro; R Quintana; Angustias Page; Angel Ramirez; A Bravo; M L Casanova; CYLD regulates keratinocyte differentiation and skin cancer progression in humans. *Cell Death & Disease* **2011**, *2*, e208-e208, 10.1038/cddis.2011.82.
23. Marcela Bolontrade; M C Stern; R L Binder; J C Zenklusen; I B Gimenez-Conti; C J Conti; Angiogenesis is an early event in the development of chemically induced skin tumors. *Carcinogenesis* **1998**, *19*, 2107-2113, 10.1093/carcin/19.12.2107.
24. Ramin Massoumi; Katarzyna Chmielarska; Katharina Hennecke; Alexander Pfeifer; Reinhard Fässler; Cyld Inhibits Tumor Cell Proliferation by Blocking Bcl-3-Dependent NF-κB Signaling. *Cell* **2006**, *125*, 665-677, 10.1016/j.cell.2006.03.041.

25. M M Chaturvedi; B Sung; V R Yadav; R Kannappan; B B Aggarwal; NF-κB addiction and its role in cancer: ‘one size does not fit all’. *Oncogene* **2010**, *30*, 1615-1630, 10.1038/onc.2010.566.
26. Le-Meng Zhang; Jing-Jing Zhou; Chun-Lei Luo; CYLD suppression enhances the pro-inflammatory effects and hyperproliferation of rheumatoid arthritis fibroblast-like synoviocytes by enhancing NF-κB activation. *Arthritis Research & Therapy* **2018**, *20*, 219, 10.1186/s13075-018-1722-9.
27. Agnieszka Kobielak; Elaine Fuchs; Links between α-catenin, NF-κB, and squamous cell carcinoma in skin. *Proceedings of the National Academy of Sciences* **2006**, *103*, 2322-2327, 10.1073/pnas.0510422103.
28. Bastian Hoesel; Johannes A Schmid; The complexity of NF-κB signaling in inflammation and cancer. *Molecular Cancer* **2013**, *12*, 86-86, 10.1186/1476-4598-12-86.
29. Alberto Mantovani; Paola Allavena; Antonio Sica; Frances Balkwill; Cancer-related inflammation. *Nature* **2008**, *454*, 436-444, 10.1038/nature07205.
30. M. Llanos Casanova; Fernando Larcher; Benito Casanova; Rodolfo Murillas; Mª Jesús Fernández-Aceñerp; Concepción Villanueva; Jesús Martínez-Palacio; Axel Ullrich; Claudio J Conti; Jose Luis Jorcano; et al. A critical role for ras-mediated, epidermal growth factor receptor-dependent angiogenesis in mouse skin carcinogenesis.. *Cancer Research* **2002**, *62*, 3402-07, .
31. Josefa P. Alameda; Mª Jesús Fernández-Aceñerp; Rita M. Quintana; Angustias Page; Ángel Ramírez; Manuel Navarro; M. Llanos Casanova; Functional Inactivation of CYLD Promotes the Metastatic Potential of Tumor Epidermal Cells. *Journal of Investigative Dermatology* **2013**, *133*, 1870-1878, 10.1038/jid.2013.76.
32. Marie Anne O’Donnell; Eva Pérez-Jiménez; Andrew Oberst; Aylwin Ng; Ramin Massoumi; Ramnik Xavier; Douglas Green; Adrian T. Ting; Caspase 8 inhibits programmed necrosis by processing CYLD. *Nature* **2011**, *13*, 1437-1442, 10.1038/ncb2362.
33. Ines Müller; Elwira Strozyk; Sebastian Schindler; Stefan Beissert; Htoo Zarni Oo; Thomas Sauter; Philippe Lucarelli; Sebastian Raeth; Angelika Hausser; Nader Al Nakouzi; et al. Cancer Cells Employ Nuclear Caspase-8 to Overcome the p53-Dependent G2/M Checkpoint through Cleavage of USP28. *Molecular Cell* **2020**, *77*, 970-984.e7, 10.1016/j.molcel.2019.12.023.
34. Gang Liang; Kristofer Ahlqvist; Rajeswararao Pannem; Guido Posern; Ramin Massoumi; Serum Response Factor Controls CYLD Expression via MAPK Signaling Pathway. *PLOS ONE* **2011**, *6*, e19613, 10.1371/journal.pone.0019613.
35. Eva M. Schindler; Anna Hindes; Erin L. Gribben; Carole J. Burns; Yan Yin; Meei-Hua Lin; Robert J. Owen; Gregory D. Longmore; Grace E. Kissling; J. Simon C. Arthur; et al. p38δ Mitogen-Activated Protein Kinase Is Essential for Skin Tumor Development in Mice. *Cancer Research* **2009**, *69*, 4648-4655, 10.1158/0008-5472.can-08-4455.
36. Alexi Kiss; Aaron C. Koppel; Joanna Anders; Christophe Cataisson; Stuart H. Yuspa; Miroslav Blumenberg; Tatiana Efimova; Keratinocyte p38δ loss inhibits Ras-induced tumor formation, while systemic p38δ loss enhances skin inflammation in the early phase of chemical carcinogenesis in mouse skin.. *Molecular Carcinogenesis* **2015**, *55*, 563-74, 10.1002/mc.22303.
37. Alexi Kiss; Aaron C. Koppel; Joanna Anders; Christophe Cataisson; Stuart H. Yuspa; Miroslav Blumenberg; Tatiana Efimova; Keratinocyte p38δ loss inhibits Ras-induced tumor formation, while systemic p38δ loss enhances skin inflammation in the early phase of chemical carcinogenesis in mouse skin.. *Molecular Carcinogenesis* **2015**, *55*, 563-74, 10.1002/mc.22303.
