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Review Article

The expression of tumor suppressor CYLD in specific neoplasias

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Abstract

The tumor suppressor CYLD is a deubiquitinating enzyme that acts as a negative regulator of several signaling pathways including NF-kappa-B and MAPK activation pathways, by affecting the function of important mediators. CYLD regulates several cellular processes such as immune responses, inflammation, cell survival, proliferation and differentiation. The tumor suppressor function of CYLD seems to be cell-type specific and it is important to delineate its involvement in the homeostasis of specific tissues in order to understand and exploit its role in oncogenesis . In this review, we summarize existing data on the expression and alterations of CYLD in certain types of lung and colorectal neoplasias.

Keywords: CYLD, lung cancer, colorectal cancer, NFκB, MAPK

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Introduction

The cylindromatosis gene Cyld codes for a 956 amino-acid protein (CYLD), which acts as a tumor suppressor and is a critical regulator of many cellular processes, such as immune responses, inflammation, cell survival, proliferation and differentiation. CYLD is a deubiquitinating enzyme, that specifically cleaves 'Lys-63'- and linear 'Met-1'-linked polyubiquitin chains. CYLD is mainly implicated on NF-kappa-B (NF-κB) and MAPK signaling and cytoskeletal dynamics. The catalytic deubiquitinating domain of CYLD is located at the carboxyl-terminal region of the protein, and three CAP-Gly domains are located within the CYLD amino terminal region, two of which are capable of interacting with microtubules (Yang & Zhou, 2016). These interactions affect cell migration and cell cycle (Wickstrom et al., 2010; Yang et al., 2014; Stegmeier et al., 2007). CYLD is mostly localized in the cytoplasm and in the perinuclear spaces of multiple cell types. Many data based on RNA transcript at Genecards.org indicate that CYLD is expressed in low levels in a large percentage of somatic cells, with immune cells expressing more of it (Genecards.org). Loss of CYLD has been linked to carcinogenesis to of several cell types (Genecards.org).

CYLD and signal transduction

CYLD regulates signaling pathways associated with cell division, cell survival and defense mechanisms. In particular, it inhibits the signal transduction pathways of NF-κB, JNK, p38, Akt and Wnt (Chowdhury et al., 2008). Some of the molecules targeted by CYLD for deubiquitination are the RIPK1, TRAF2, TRAF6, TAK1 and NEMO proteins (Trompouki et al., 2003). Moreover, CYLD deubiquitinates the protein BCL-3, preventing it from entering the nucleus (Massoumi et al., 2006). It should be stressed out that the effect of CYLD on the various pathways and substrates mentioned, depends on the cell type. Therefore, the analysis of the molecular mechanism of action of CYLD should be performed in every cell type in order to be reliable.

CYLD and neoplasias

The tumor suppressor gene CYLD has attracted the interest of the scientists recently, due to its association with many types of cancers including hepatocellular carcinoma, colorectal adenocarcinoma, breast cancer and non-small cell lung cancer (Mathis et al., 2015). Downregulated expression and inactivating mutations within Cyld gene have been observed in several types of human malignancies (Rajan & Ashworth, 2015). In this review, we have focused on lung and colon neoplasias.

CYLD and lung neoplasias

Squamous-cell carcinoma (SCC) of the lung is the 2nd most common type of malignant non-small cell epithelial tumors of the lung (25% of lung cancers). SCC usually is located in the center of the lung, primarily in the larger bronchi. This type of cancer is highly linked with smoking when compared to other types of non-small cell lung cancer. According to the world health organization (WHO) there are 4 subtypes of SCC which are: papillary, clear cell, small cell and basaloid (Travis et al., 1999), NIH non-small cell lung cancer treatment). Lung adenocarcinoma is the most common type of lung cancer (40% of lung cancers). It is characterized by frequent histologic heterogeneity. This type of lung cancer is associated with smoking and is most common to younger women and the Asian population. Lung adenocarcinoma is

also classified into several variants such as: well-differentiated fetal adenocarcinoma, mucinous (colloid) adenocarcinoma, mucinous cystadenocarcinoma, signet ring adenocarcinoma and clear cell adenocarcinoma. (Travis et al., 1999; NIH non-small cell lung cancer treatment). The evidence supporting the involvement of CYLD in non-small cell lung cancer includes its suppression in lung cancer samples compared to normal tissue (Zhong et al., 2007; Zhong et al., 2007; Deng et al., 2012; Lin et al., 2016). In addition, mice which express a mutant non-functional form of CYLD (CYLD Δ9), are unable to develop a mature and functional lung, suggesting an important role for CYLD in development and differentiation of the lung (Trompouki et al., 2009). Furthermore, immunohistochemistry data from patients with lung cancer, extracted from protein atlas (www.proteinatlas.org) showed that CYLD was undetected in 8/11 samples, using the CYLD specific antiboby HPA050095. Furthermore 3/11 samples were stained with low intensity. The staining was mainly cytoplasmic. Additionally, RNA sequencing data showed that 43% of the patients with low expression of CYLD (lower than the expression cutoff: 4.47) had a 5-year survival rate and 47% with high expression (higher than 4.47) had the same 5-year survival. As it is suggested from the immunohistochemistry data, loss of CYLD is connected to carcinogenesis. However, due to the very small size of the sample, we cannot safely conclude on the connection among CYLD expression, the progress of carcinogenesis and the patient's survival.

To further investigate the involvement of Cyld gene in lung cancer, we extracted data from cBioPortal (www.cbioportal.org). As it is shown in table 1, CYLD mutations were identified in 13 patients out of 487 with lung squamous cell carcinoma. This corresponds to a percentage 3.9%. 3 out of 13 were due truncations due to alternative splicing, 1 was nonsense, due to stop codon, and 9 were missense (the aminoacid change is included in Table 1). 3/13 truncations and 5/13 missense mutations are located in the catalytic domain at the C-terminus.

Similarly, CYLD mutations were identified in 10 patients out of 566 with lung adenocarcinoma. This corresponds to a percentage 3.1% (Table 2). 2 were nonsense mutations and 8 were missense . Most of them are located in the catalytic domain at the C-terminus.

CYLD and colitis-associated colorectal carcinogenesis

Colorectal cancer (CRC) ranks as the third most common malignancy in both genders; its most common histologic type is adenocarcinoma (Barresi et al., 2015; Siegel et al., 2017). Regarding its incidence there has been a decline in the last few years for people aged above 50, whereas for the younger people, due to better screening methods, there has been an increase by 22%. As far as CRC survival is concerned, an improvement has been noted, since 5 year survival is now reaching 66% for colon and 68% for rectal cancer; this is due mostly to screening and better treatment modalities (Siegel et al., 2017). Most of the cases are sporadic (75%) with the rest being either familial (20%) or

Table 1: CYLD mutations that were identified in 13 patients out of 487 with lung squamous cell carcinoma (the data were extracted from cBioPortal, www.cbioportal.org).

Protein Change	**Mutation Type**
X784_splice	**Splice**
X747_splice	**Splice**
S809*	**Nonsense**
X305_splice	**Splice**
G71V	**Missense**
V864F	**Missense**
P682T	**Missense**
P806S	**Missense**
S615C	**Missense**
L230F	**Missense**
G341V	**Missense**
P337S	**Missense**
C817Y	**Missense**

inherited (Mármol et al., 2017). Various translocations and mutations have been found that influence important pathways such as WNT, MAPK/P13k and TGF-β , thus leading to carcinogenesis (Mármol et al., 2017). Deregulation of CYLD expression has been associated with several intestinal pathologies, including inflammatory bowel disease like Crohn's disease and ulcerative colitis in humans (Cleynen et al., 2014). Furthermore, mice with *Cyld* deficiency have been found to be sensitized to colitis-associated cancer development, i.e., they exhibit a dramatic increase in the incidence and multiplicity of intestinal tumors induced by azoxymethane (AOM) and dextran sodium sulfate (DSS) (Zhang et al., 2006). In addition, intestinal epithelial cells (IEC) of mice with *Cyld* deficiency developed larger and significantly more adenomatous polyps than normal mice, when treated with AOM and DSS (Karatzas et al., 2016; Fernández-Majada et al., 2016).The signaling pathways that mediate the tumor suppressing role of CYLD in IECs are as yet unknown. Furthermore, immunohistochemistry data from patients with colorectal adenocarcinoma, extracted from "the human protein atlas" database

Table 2: CYLD mutations that were identified in 10 patients out of 566 with lung adenocarcinoma (the data were extracted from cBioPortal, www.cbioportal.org).

Protein Change	**Mutation Type**
Q69*	**Nonsense**
K114T	**Missense**
A305S	**Missense**
G360V	**Missense**
E390*	**Nonsense**
E390V	**Missense**
P464S	**Missense**
R540M	**Missense**
G593D	**Missense**
G900V	**Missense**

Table 3: CYLD mutations that were identified in 17 patients out of 549 with colon and rectal adenocarcinoma (the data were extracted from cBioPortal, www.cbioportal.org).

Cancer Type	**Protein Change**	**Mutation Type**
Colon Adenocarcinoma	***N722Mfs*13***	**FS del**
Colon Adenocarcinoma	***N722Mfs*13***	**FS del**
Colon Adenocarcinoma	***N722Mfs*13***	**FS del**
Colon Adenocarcinoma	***G160R***	**Missense**
Colon Adenocarcinoma	***P42L***	**Missense**
Colon Adenocarcinoma	***K658R***	**Missense**
Colon Adenocarcinoma	***E447D***	**Missense**
Colon Adenocarcinoma	***V534A***	**Missense**
Colon Adenocarcinoma	***L939F***	**Missense**

Cancer Type	Protein Change	Mutation Type
Rectal Adenocarcinoma	***G47****	**Nonsense**
Rectal Adenocarcinoma	***D893N***	**Missense**
Rectal Adenocarcinoma	***T340I***	**Missense**
Rectal Adenocarcinoma	***E684K***	**Missense**
Rectal Adenocarcinoma	***T778K***	**Missense**

(www.proteinatlas.org) showed that CYLD was undetected in 4/10 samples, using the CYLD specific antiboby HPA050095. Furthermore 5/10 samples were stained with moderate intensity and 1/10with low intesity. The staining was mainly cytoplasmic. Additionally, RNA sequencing data showed that 62% of the patients with low expression of the protein (lower than the expression cut off: 1,57) had a 5-year survival rate and 60% with high CYLD expression (higher than 1,57) had the same 5-year survival. As it is suggested from the immunohistochemistry data, loss of CYLD is connected to carcinogenesis in humans. However, due to the very small size of the sample, we cannot safely conclude on the connection among CYLD expression, the progress of carcinogenesis and the patient's survival. Similarly to the lung data, we looked into mutations in the *Cyld* gene. CYLD mutations were identified in 17 patients out of 549 with colon and rectal adenocarcinoma (Table 3, the data were extracted from cBioPortal, www.cbioportal.org). 9 were identified in colon adenocarcinoma, including 3 deletions, and 5 missense. Similarly, 5 were identified in rectal adenocarcinoma, 1 nonsense and 4 missense.

Conclusion

The tumor suppressor CYLD was discovered in the year 2000. Since then significant and crucial progress has occured towards understanding the anti-tumor and the physiological function of CYLD, as well as discovering the molecular mechanism of its signaling action. CYLD and its genetic mutations are associated with skin appendage tumors. However, it has been found that mutations and reduced expression of CYLD also exist in other types of tumors. It has been shown that CYLD has a critical role mainly in the regulation of the NF-κB and MAPK pathways, thus playing an important role on the growth and survival of different types of cancer cells. The studies in animal models have been crucial in further understanding the physiological functions and the tumor suppressor action of CYLD. Even though there have been some discrepancies in the findings, it is generally accepted that CYLD has an important role in the regulation of the immune response, the inflammation process, the development of immune and non-immune cells, apoptosis, tumorigenesis, mitosis and cell migration. Further studies are required to elucidate its cell type-dependent and stimuli-dependent roles.

Understanding how CYLD regulates normal biological functions and how its expression can be augmented could lead to new therapeutic approaches. Towards this direction, previous

studies have shown that expression of CYLD can be increased after treatment with histone deacetylase inhibitors (Kotantaki and Mosialos, 2016) and TZD (Pseftogas et al, 2017) in liver and breast cancer cell lines respectively. It will be significant to screen for drugs that can augment CYLD expression in different tumor cell lines. A better understanding of CYLD regulation is important for using this tumor suppressor as a new anti-cancer therapeutic target.

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