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Deubiquitinating Enzymes as Cancer Biomarkers: New Therapeutic Opportunities?

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ABSTRACT

Cancer remains a life-threatening disease and accounts for the major mortality rates worldwide. The practice of using biomarkers for early detection, staging, and customized therapy may increase cancer patients' survival. Deubiquitinating enzymes (DUBs) are a family of proteases that remove ubiquitin tags from proteins of interest undergoing proteasomal degradation. DUBs play several functional roles other than deubiquitination. One of the important roles of DUBs is regulation of tumor progression. Several reports have suggested that the DUB family members were highly-elevated in various cancer cells and tissues in different stages of cancer. These findings suggest that the DUBs could be used as drug targets in cancer therapeutics. In this review, we recapitulate the role of the DUB family members, including ubiquitin-specific protease, otubain protease, and important candidates from other family members. Our aim was to better understand the connection between DUB expression profiles and cancers to allow researchers to design inhibitors or gene therapies to improve diagnosis and prognosis of cancers.

INTRODUCTION

Cancer is one of the major diseases causing death globally, accounting for 8.2 million deaths in 2012 (1). Recent advances in molecular and cellular biology have played important roles in understanding cancer and breakthroughs that have been being translated into therapy. Because of these recent developments, genes that are involved in cancer are being unraveled (2, 3). Most of the known cancer genes were originally identified by genetic evidence. A protein that is encoded by a cancer gene typically regulates cell proliferation and differentiation and eventually leads to cell death or apoptosis. Mutations that lead to oncogenesis typically occur in genes that mediate DNA repair mechanisms (4).

Ubiquitin proteasome pathway

The post-translational attachment of ubiquitin is a modification that can determine a protein's fate. While ubiquitin itself is a small conserved protein, its covalent conjugation to protein substrates and to other ubiquitin molecules is a tightly-controlled process involving complex cellular machinery. Perhaps the most prominent and well-known function of ubiquitin is to target a protein for degradation by the 26S proteasome. Degradation can be accomplished via an isopeptide bond formation between the carboxy-terminal glycine (Gly) site on the ubiquitin and an ϵ -amino group of the lysine (Lys) side chains of a protein substrate. The ubiquitin-substrate system is further diversified via the process of polyubiquitination, during which a ubiquitin molecule's C-terminal Gly is

conjugated with one of the seven Lys residues on another ubiquitin (Lys6, Lys11, Lys27, Lys29, Lys33, Lys48, or Lys63) or with the N-terminus to form linear chains.

The ubiquitin-proteasome protein degradation pathway is comprised of ubiquitin, a three-enzyme ubiquitination complex, the intracellular protein ubiquitination targets, and the proteasome that is the organelle of protein degradation, as well as ubiquitin-activating enzymes (E1), ubiquitin-conjugating enzymes (E2), and ubiquitin ligases (E3).

Deubiquitinating enzymes

Deubiquitinating enzymes (DUBs) are proteases that reverse protein ubiquitination, a process which is significant for normal homeostasis. DUBs have four distinct mechanisms of action: 1) processing of ubiquitin precursors, 2) recycling of ubiquitin molecules during ubiquitination, 3) cleavage of polyubiquitin chains, and 4) reversal of ubiquitin conjugation (Figure 1). DUBs regulate several cellular functions, including proteasome-dependent and lysosome-dependent proteolysis, gene expression, cell cycle progression, chromosome segregation, kinase activation, apoptosis, localization, DNA repair, maintenance of stemness, spermatogenesis, and degradation of signaling intermediates (5–10).

Approximately 100 DUBs are encoded in the human genome [10]. Based on the organization of the catalytic domain, DUBs are classified into distinct families, the vast majority of which are cysteine proteases. These include ubiquitin-specific proteases (USPs), ubiquitin C-terminal hydrolases (UCHs), ovarian tumor proteases (OTUs), Machado-Joseph disease proteases (MJDs), Jab1/Mov34/Mpr1 (JAMM) metalloproteases, and the recently-discovered MIU-containing novel DUB family, (MINDY) proteases (11).

UBIQUITIN-SPECIFIC PROTEASE FAMILY

USP2

USP2a, an isoform of USP2, is an androgen-regulated DUB that deubiquitinates the antiapoptotic proteins such as fatty acid synthase, Mdm2, and MdmX (12). USP2a expression is increased in glioma cells compared to normal brain tissues, which suggests that USP2a may correlate with malignant glioma progression, and therefore may be an effective marker for glioma prognosis (13). Furthermore, USP2 plays a role in tumor metastasis by modulating the activity or expression of MMP2, suggesting its use as a potential breast cancer marker (13).

USP4

USP4 has been strongly implicated in the regulation of tumor metastasis in breast cancer (14), liver cancer (15), and colorectal cancer (16). USP4 is significantly increased in melanoma and plays an oncogenic role by simultaneously inhibiting stress-induced cell apoptosis and promoting tumor metastasis (17). USP4 is an important protein that facilitates hepatocellular carcinoma (HCC) progression by stabilizing CYPA through deubiquitination and activating MAPK/Erk1/2 signaling pathways, indicating that USP4 may act as a novel marker to predict prognosis and present a therapeutic opportunity for HCC (18). In breast cancer, USP4 promotes the migration and invasion of breast cancer cells via RLX-mediated TGF- β /Smad2/MMP-9 pathways, providing an attractive target for breast cancer therapy (19). The above results suggested that overexpression of USP4 in various

cancers is due to the stabilization of other oncogenes in the respective cancer types. Thus, targeting USP4 as a biomarker could be useful for the early diagnosis of cancer.

USP5

USP5 acts as a exopeptidase that hydrolyzes isopeptide bonds in poly-ubiquitin from their free carboxy-terminal ends to produce monoubiquitin (20). USP5 knockdown suppressed cell proliferation, migration, and drug resistance and induced apoptosis, while USP5 overexpression promoted colony formation, migration, drug resistance, and tumorigenesis (21). USP5 plays a critical role in hepatocarcinogenesis through inactivation of the p14-p53 signaling pathway contributing to tumorigenesis and drug resistance, which provides a clue that USP5 could be a potential therapeutic target for HCC (22). In pancreatic cancer, USP5 plays a critical role in tumorigenesis and progression by stabilizing the FoxM1 protein, showing therapeutic potential against pancreatic cancer (23).

USP7

USP7, also known as herpes-associated ubiquitin-specific protease (HAUSP), was originally identified as an ubiquitin-specific protease that binds to a viral-encoded protein, called Vmw110 (24). HAUSP protein can bind to the herpes simplex virus type 1 (HSV-1) regulatory protein, which is known as infected cell polypeptide (ICPO). In epithelial ovarian cancer (EOC), USP7 and MARCH7 proteins are differentially expressed and the combination of USP7 and MARCH7 expression may function as promising biomarkers for EOC prognosis (25). HCC is one of the most dominant cancer types in the world. High

expression of USP7 mRNA and protein levels in HCC tissues compared to normal liver samples has been reported (26). Cell-based assays have suggested that USP7 expression confers cell proliferation, migration, and invasion capabilities. These data suggest that USP7 could be a novel independent prognostic marker for HCC. Recently, a study suggested that USP7 deubiquitinates Ki-67, and thereby promotes cell proliferation in non-small-cell lung cancer (NSCLC) (27). Here, both Ki-67 and USP7 were expressed in NSCLC cells. Statistical data revealed a strong correlation between USP7 and Ki-67 levels. In contrast, siRNA targeting USP7 increased the ubiquitination of Ki-67 and led to delayed tumor growth. The above evidence suggests that USP7 could be an important therapeutic target in various cancer types.

USP8

USP8 belongs to the USP superfamily of DUBs targeting several substrates (28), including smoothened (29), frizzled (30), neuregulin receptor degradation protein-1, and receptor tyrosine kinase. Recently, the expression profile of USP8 in cervical squamous cell carcinoma (CSCC) has been studied (31). USP8 was upregulated in CSCC tissue samples compared to non-cancerous cervical tissues. Also, high expression of USP8 was associated with tumor stage and was recognized as an independent prognostic marker for CSCC. Elevated levels of USP8 led to cell proliferation, migration, and invasion of CSCC cell lines. Thus, USP8 could be a therapeutic and diagnostic target in CSCC patients.

USP10

USP10, also known as UBPO, is a protein consisting of 798 amino acids and was originally discovered as a DUB that interacts with the Ras-GAP SH₃ domain-binding protein (32). Increased USP10 expression has been detected in some breast cancer and glioblastoma samples. Overexpression of USP10 has been associated with poor prognosis for glioblastoma multiforme patients, while decreased USP10 has been observed in gastric cancer tissues, and its downregulation has been associated with invasion, metastasis, and poor prognosis of gastric cancer. Current studies have also shown that USP10 suppressed proliferation and growth of pancreatic cancer cells. Therefore, USP10, as a novel DUB, has a crucial role in various pathological processes of tumors. In gastric cancer (GC), clinical samples and cell lines showed low-level expression of USP10, and negative USP10 expression was associated with a marked propensity toward gastric wall invasion, lymph node metastasis, highly malignant biological behavior, and poor survival. USP10 identification in GC can potentially serve as a new prognostic indicator predicting the treatment outcome for GC patients.

USP22

USP22 is a novel DUB that has been related to cell cycle progression, therapy resistance, and metastasis. The expression frequency of USP22 was extremely high in HCC compared to normal liver tissues (31). Elevated levels of USP22 represented poor HCC patient survival and have also been associated with greater mortality in patients with advanced tumor stages, shown by Kaplan-Meier analysis. As revealed by multivariate

analyses, USP22 is a self-regulating prognostic marker in HCC. Several other researchers have reported that USP22 was overexpressed in salivary duct carcinoma (33) and esophageal squamous cell carcinoma (34). The above findings indicate that high USP22 expression might be an important factor in tumor progression and may serve as an independent molecular marker.

USP32

USP32 is a highly-conserved and uncharacterized gene, located on the 17q23.1-17q23.2 chromosomal band (35). USP32 was present in 22% of primary breast cancer tumors compared to non-cancerous mammary tissues, and 50% of breast cancer cell lines. Endogenously, USP32 was highly-elevated in the MCF7 cell line, and no mutation was detected in this cell line, indicating that the wild-type gene was overexpressed. Additionally, USP32 has a role in human small cell lung cancer (SCLC) (36). USP32 is highly-expressed in SCLC tissue samples compared to normal tissues. During the disease aggravation stage, USP32 has been positively correlated with SCLC expression. On the other hand, downregulation of USP32 *in vitro* caused reduced migration and proliferation rates of SCLC cells. Also, this downregulation arrested the cells at the G0/G1 phase by elevating p21 and decreasing CDK4-cyclin D1 complex levels. Cleaved caspase-3 and cleaved-PARP were activated when the USP32 gene was silenced, eventually leading to apoptosis by altering the epithelial-mesenchymal transition. Overall, USP32 could be a potential target for breast and lung cancers.

OTUBAIN PROTEASE FAMILY

OTUB1

The OTU domain-containing ubiquitin aldehyde-binding protein 1 (OTUB1) belongs to the OTU DUB family and is reported to be involved in various malignancies (37-40). Recently, the role of OTUB1 in human gliomas has been elucidated (41). Immunoblot and immunohistochemical experiments validated that glioma tissues overexpress OTUB1 genes and statistical studies showed that the expression pattern of OTUB1 was highly-linked to the WHO grades of the gliomas. On the other hand, downregulation of OTUB1 was linked to poor migration and also elevated EMT-related protein E-cadherin expression. Thus, OTUB1 might be involved in the regulation of ECM stability. The above results suggested that OTUB1 could be an important cancer marker in gliomas and other malignancies and could be a potential target for successful cancer therapy.

A20

A20 is a DUB that was originally found to be involved in autoimmunity and inflammation (42). However, a recent study proposed that A20 is highly involved in cancer metastasis (43). Here, A20 overexpression leads to metastasis of basal-like breast cancer by monoubiquitinating Snail1. In human basal-like breast cancers, A20 was significantly overexpressed and accounted for cancer metastasis. Additionally, A20 mediates TGF- β 1-induced EMT of breast cancers by monoubiquitylating Snail1. Reports also suggested that

the transient knockdown of A20 displayed decreased lung cancer metastasis in orthotopic breast cancer models and mouse xenografts.

DUB INHIBITORS

A number of reports have described the identification and utility of small molecule DUB inhibitors as anticancer agents (44). Inhibition of DUBs leads to cellular changes, such as (i) aggregation of polyubiquitinated protein molecules, (ii) reduction in the group of monomeric ubiquitin moieties, (iii) increased rates of polyubiquitin assembly, (iv) overall reduction in DUB events, and (v) altered cellular activities, such as DUB regulation of oncoproteins (45). Generally, DUB inhibition leads to impaired proteasome function and aggregation of misfolded functional proteins, resulting in cellular toxicity and death. DUBs that control oncogenic proteins can be targeted by small molecules that inhibit deubiquitinating activity via UPS degradation, while DUBs that control tumor suppressors can be targeted by increasing the deubiquitinating activity, thus inhibiting oncogenic progression. Several studies have been carried out to design small molecule DUB inhibitors because they are simpler to design than enzyme activators, using substrate modeling and competitive inhibition (46, 47). A schematic representation of DUB inhibition on relevant pathways is depicted in Figure 2.

An extensive study was carried out to discover drugs that inhibit DUBs and resulted in the discovery of ubiquitin aldehyde (Ubal) and ubiquitin vinyl sulfone (UbVS) (48). Due

to their high molecular weights, peptidic nature, and lack of specificity, these compounds were not pharmacologically sustainable (48). The UCH family of proteins is involved in deubiquitination by removing ubiquitin from C-terminal adducts [48]. Hence, researchers have made an effort to design their inhibitors and ended up with an isatin O-acyl oximes series (48). They are competitive and capable of directly targeting the active site with minimal IC_{50} values. Basically, UCH-L1 decreases cell proliferation in neuroblastoma cells; when this inhibitor is applied, cell proliferation is elevated. Thus, the data support the anti-proliferative nature of UCH-L1 proteins. A novel proteasome-inhibitory compound has been synthesized, called b-AP15 (49). The b-AP15 small molecule specifically inhibits USP14 and UCHL5 which are associated with 19S RP. Additionally, the compound b-AP15 showed effective anti-cancer responses against other refractory cancer types. Inhibitors targeting other important DUBs are described in Table 1.

CONCLUSION

This review delivers a comprehensive report of the DUBs for cancer diagnosis and prognosis. Recent developments of cancer therapies and the promptness of their application to clinical use for various tumors validate the prospects of exploiting DUBs as targets for drug development. The expression profiles of several DUBs in different cancer types are discussed in Table 2. Moreover, these DUBs exert their function through binding to their proteins, which can be targeted. In other cases, the DUB itself seems to be an excellent drug target. More detailed information on the roles, localization, regulation, and substrates of DUBs will help researchers understand their roles in oncogenesis and clinical

applications of their inhibitors. Enhanced improvement in small molecule pharmacological development against DUBs will permit greater success in the treatment of cancer and other deadly diseases.

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Table 1. DUBs and their inhibitors in cancer therapeutics

DUB	Inhibitor(s)	Disease Indication	Stage of development	References
USP1	ML323, Pimozide	Oncology	Preclinical	(50)
USP2	ML364	Inflammation	Preclinical	(50)
USP4	Vialinin A	Inflammation and oncology	Preclinical	(51, 52)
USP5	WP1130			
	ADC-01, ADC-03	Oncology, Immuno-oncology	Preclinical	(53)
	HBX41108	Oncology, Immuno-oncology	Preclinical	(54, 55)
USP7	P5091	Oncology, Immuno-oncology	Preclinical	(56)
	P22077	Oncology, Immuno-oncology	Preclinical	(56)
USP8	9-(Ethoxyimino)-9H-indeno (1,2-b) pyra-zine-2,3-dicarbonitrile	Oncology	Preclinical	(56)
USP9X	WP1130	Oncology	Preclinical	(57)
USP10 and USP13	Spautin 1	Inflammation	Preclinical	(57)
USP11	Mitoxantrone	Oncology	Preclinical	(58)
USP14	1U1, b-AP15, VLX1570, wp1130	Neurodegeneration	Preclinical	(59)
USP20	GSK2643943A	Oncology	Preclinical	(60)
USP30	15-oxospiramilactone	Neurodegeneration	Preclinical	(61)
USP47	P5091	Cancer	Preclinical	(56)
UCH37	WP1130	Cancer	Preclinical	(57)

UCHL5	b-AP15	Neurology	Preclinical	(62)
UCHL1	LDN-57444	Cancer	Preclinical	(63)
UCHL3	LDN-57444	Cancer	Preclinical	(64)
UCHL5	TCID, b-AP15	Cancer	Preclinical	(62, 65)
UCH37	WP1130	Cancer	Preclinical	(57)

Table 2. DUBs expressed in various types of cancer

Disease	DUB	References
Gliomas	USP2a, USP22, USP44, BAP1, OTUB1	(41, 66, 67)
Breast cancer	USP2, USP22, USP37	(68, 69)
Hepatocellular carcinoma	USP4, USP5, USP11, USP22, UCHL1, A20, OTUB1	(70, 71)
Esophageal cancer	USP4	(72)
Melanoma	USP4	(72)
Pancreatic cancer	USP5	(72)
Epithelial ovarian cancer	USP7	(72)
Lung adenocarcinoma	USP8	(73, 74)
Gastric carcinoma	USP10	(75)
Endometrial cancer	USP14	(75)
Non-small cell lung carcinoma	USP17, USP22, OTUD7B, OTUD6B	(76, 77)
Renal clear cell carcinoma	USP21	(78)
Muscle invasive bladder cancer	USP18	(79)
Cervical cancer	USP22	(80)
Oral squamous cell carcinoma	USP22	(81)
Papillary thyroid carcinoma	USP22, USP33	(82, 83)
Salivary duct carcinoma	USP22	(33)
Esophageal squamous cell carcinoma	USP22	(34)

Salivary adenoid cystic carcinoma	USP22	(34)
Bladder cancer	USP28	(84)
Colorectal cancer	USP33, OTUB1, MYSM1	(85, 86)
Prostate cancer	USP39	(87)
Malignant peritoneal mesothelioma	BAP1	(88)
Triple-negative breast cancer	OTUD7B	(89)
Pancreatic ductal adenocarcinoma	UCHL5	(90)
Gastric cardiac adenocarcinoma	UCHL1	(91)
Cholangiocarcinoma	UCHL1	(91)
Aggressive multiple myeloma	UCHL1	(92)
Neuronal apoptosis	USP4, UCHL1	(93, 94)
Cardiac hypertrophy	USP4	(95)
Aneurysmal bone cyst	USP6	(96)
Pancreatic beta cells	UCHL1	(97)
Aneurysmal subarachnoid hemorrhage	UCHL1	(98)
Neuronal biomarker	UCHL1	(98)
Traumatic brain injury	UCHL1	(99)
Pancreatic neuroendocrine tumors	UCHL1	(100)

FIGURE LEGENDS

Figure 1. Various catalytic mechanisms exhibited by DUBs. DUBs can unknot ubiquitin conjugation by cleaving the bond between ubiquitin molecules and ubiquitin-target complexes, editing ubiquitin chains to remove one or more ubiquitin molecules, and finally, recycling of ubiquitin molecules in the ubiquitin-proteasome pathway.

Figure 2. DUBs involved in the regulation of oncogenic pathways. Inhibition of specific DUBs leads to decreased cancer proliferation, drug resistance, and delayed metastasis.

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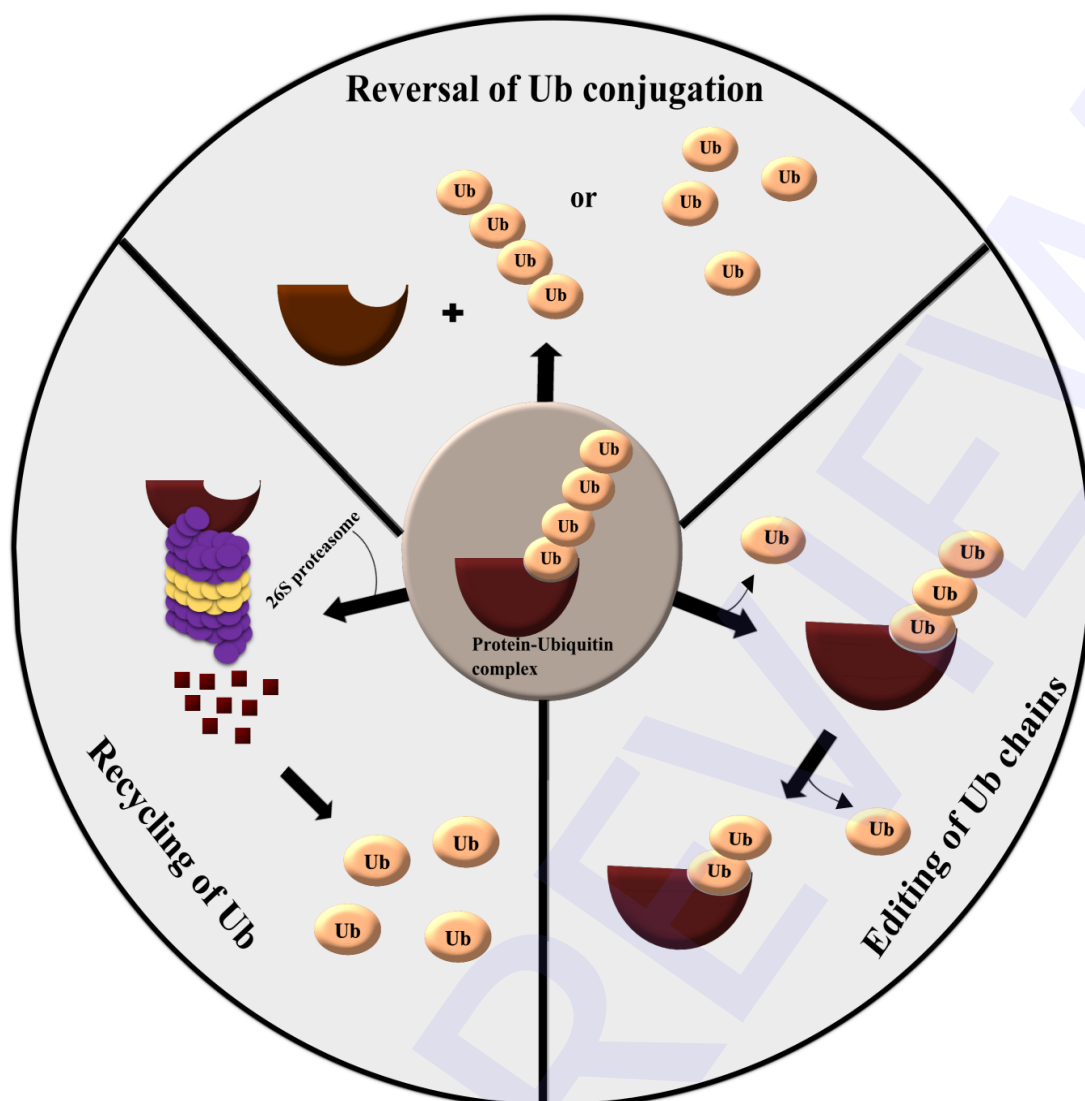


Fig. 1.

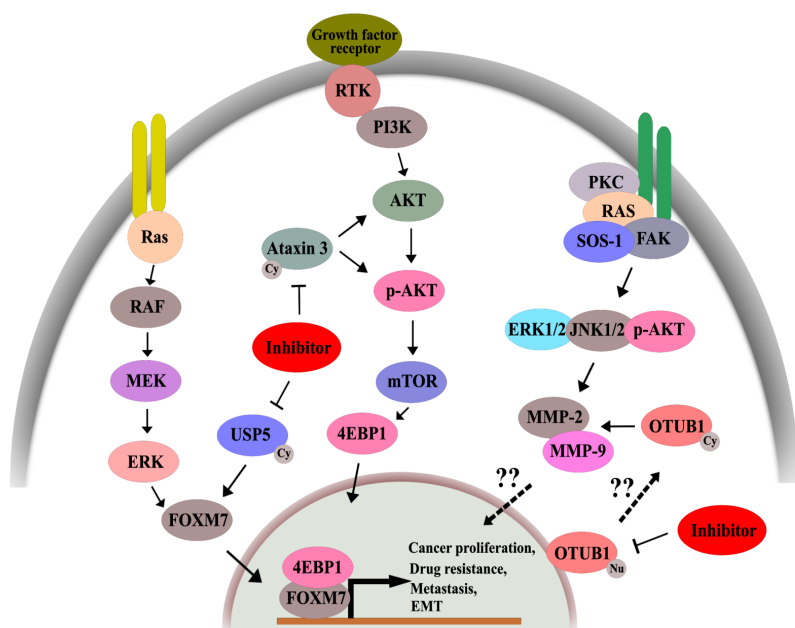


Fig. 2.