Biomarkers and Cancer Targets

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Abstract

Biomarkers are molecules that indicate normal or abnormal process taking place in your body and may be a sign of an underlying condition or disease. Various types of molecules, such as DNA (genes), proteins or hormones, can serve as biomarkers, since they all indicate something about your health. A biomarker, or biological marker, generally refers to a measurable indicator of some biological state or condition. The term is also occasionally used to refer to a substance whose detection indicates the presence of a living organism. Biomarkers are often measured and evaluated to examine normal biological processes, Biomarkers are distinct biological indicators (cellular, biochemical or molecular) of a process, event or condition that can be measured reliably in tissues, cells or fluids, and can be used to detect early changes in a patient's health. Some examples of biomarker include blood cholesterol a well-known biomarker of risk for, Biomarker is short for biological marker, and is used as an indication that a biological process in the body has happened or is ongoing. While some biomarkers are used to show that the body has been exposed to a chemical toxin or other environmental impact - most associate biomarkers with medicine.

A biological molecule found in blood, other body fluids, or tissues that is a sign of a normal or abnormal process, or of a condition or disease. A biomarker may be used to see how well the body responds to a treatment for a disease or condition. NIH Biomarkers Definitions Working Group: "A characteristic that is objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes, or pharmacologic responses to a therapeutic intervention." World Health Organization: "Any substance, structure, or process that. Biomarkers are characteristics of the body that you can measure. So your blood pressure is actually a biomarker. Biomarkers are very important to medicine in general. We're all used to going to the doctor and getting all our test results, right, and even imaging x-ray results or CAT scans.
Keywords: Biomarkers / Cancer Cells / Cancer Therapy / Cancer Targets

Cancer is a disease of the cell (Doll R, Peto R, 1981). This rather simple statement implies an enormous complexity when attempting to identify efficacious anticancer agents. One of the major issues associated with anticancer research is that traditional target-directed strategies are confronted with the essentiality of the function of the target in healthy cells. Inevitably, targeting proteins that have essential functions are likely to lead to chemical entities with narrow therapeutic windows and significant toxic effects. An additional challenge is the unstable epigenetic and genetic status of cancer cells, undergoing multiple mutations, gene copy alterations, and chromosomal abnormalities that have a direct impact on the efficacy of anticancer agents at different stages of the disease (Bernstein H et.al (2013). All these aspects make cancer drug discovery extremely difficult and have led to poor clinical approval success rates compared to other therapeutic areas.

Therefore, individualized therapy is paramount for improving of cancer treatment. The development of rationalized and individualized therapy is reliant on the identification of the specific biomarkers, validation of the biomarkers to identify the therapeutic targets, and drug development against the identified.

A Cancer marker or tumor marker is a biomarker found in blood, pee, or body tissues that can be raised by the proximity of at least one sorts of development. There are different tumor markers, each illustrative of a particular alignment. In addition to their use in cancer medicine, biomarkers are often used throughout the cancer drug discovery process. For instance, in the 1960s, researchers discovered the majority of patients with chronic myelogenous leukemia possessed a particular genetic abnormality.

Introduction

Cancer is a gathering of sicknesses including irregular cell development with the possibility to attack or spread to different parts of the body. These stand out from benevolent tumors, which don't spread to different parts of the body. Possible signs and side effects incorporate a bump, strange dying, delayed hack, unexplained weight reduction, and an adjustment in gut movements. Tobacco use is the cause of about 22 % of cancer deaths. Another 10 % are due to obesity, poor diet, lack of physical activity, and excessive drinking of alcohol. Other factors include certain infections, exposure to ionizing radiation and environmental pollutants. In the developing world, 15 % of cancers are due to infections such as Helicobacter pylori, hepatitis B, hepatitis C, human papillomavirus infection, Epstein–Barr virus and human immunodeficiency virus. , Colorectal cancer, Non-Hodgkin lymphoma, Prostate cancer, Lung cancer, stomach cancer.

Cancer starts when cells change abnormally. Cancer is when abnormal cells divide in an uncontrolled way. Some cancers may eventually spread into other tissues. There are more than 200 different types of cancer. 1 in 2 people in the UK will get cancer in their lifetime. Thanks to research many people are cured.

Cancer grows as cells multiply over and over. Cancer starts when gene changes make one cell or a few cells begin to grow and multiply too much. This may cause a growth called a tumor.
Some cancers can spread to other parts of the body. A primary tumor is the name for where a cancer starts. Cancer can sometimes spread to other parts of the body – this is called a secondary tumor or a metastasis. Cancer and its treatments can affect body systems, such as the blood circulation, lymphatic and immune systems, and the hormone system.

Most cancers start due to gene changes that happen over a person’s lifetime. More rarely cancers start due to inherited faulty genes passed down in families. Genes, DNA and cancer. Genes and inherited cancer risk. Cancer can sometimes come back. Many cancers are cured. But in some people cancer can return. Some cancers can’t be cured but treatment is often able to control them for some years.

Cancers are divided into groups according to the type of cell they start from. They include Carcinomas, Lymphomas, Leukaemias, Brain tumours, Sarcomas.

Stages and grading of cancer Staging and grading give an idea of how quickly a cancer may grow and which treatments may work best. The stage of a cancer means how big it is and whether it has spread. Grading looks at how abnormal the cancer cells (Stewart BW, 2014).
Many tumor types are associated with genetic changes in the retinoblastoma pathway, leading to hyperactivation of cyclin-dependent kinases and incorrect progression through the cell cycle. Small-molecule cyclin-dependent kinase inhibitors are being developed as therapeutic agents. Of these, flavopiridol and UCN-01 are being explored in cancer patients in phase I and phase II clinical trials, both as single agents and in combination with conventional chemotherapeutic agents. The present article discusses the mechanisms of action of flavopiridol and UCN-01 as well as the outcome of clinical trials with these novel agents.

During progression through the phases of the cell cycle (G₁, S [DNA synthesis], G₂, M [mitosis]), DNA is duplicated and the chromosome sets are distributed evenly over the two daughter cells. To ensure accuracy of the cell-cycle progression, cells need to go through several pauses or “checkpoints.” At the checkpoint in late G₁, the cell either exits to G₀ and becomes quiescent or commits to the cell cycle. The G₂ checkpoint allows the cell to repair DNA damage before entering mitosis. Cell-cycle progression is regulated by cyclin-dependent kinases (cdks), a family of serine/threonine kinases, which during G₁ progressively phosphorylate the retinoblastoma (Rb) protein. Upon phosphorylation, Rb is inactivated and releases transcription factors of the E2F family, which subsequently induce transcription of genes needed for S-phase entry. Cdk4 are positively regulated by cyclins with which they form holoenzymes. In addition, cdkks are positively regulated by cdk7, which, in a complex with cyclin H, phosphorylates cdks at threonine 160/161. Negative regulation of cdks is performed by two families of cdk inhibitors. The INK4a family of cdk inhibitors includes p16INK4a, p15INK4b, p18INK4c, and p19INK4d and specifically inhibits cdk4 and cdk6, which form complexes with D-type cyclins. In contrast, the Cip/Kip family consists of p21Cip1, p27Kip1, and p57Kip2 and inhibits most cdks. Cdk1 (cdc2), the cdk responsible for the G₂/M transition, forms complexes with cyclin B and regulation of this cdk involves reversible phosphorylation at tyrosine 15 and threonine 14.

In human cancer, the Rb pathway (cyclin D1/cdk4/ p16INK4a/Rb) frequently is nonfunctional. Although few human tumors contain a mutation of the Rb gene itself, the majority of human malignancies have derangement in Rb function due to “hyperactivation” of cdks due to an increase in the catalytic subunit (cdk) or the cofactors (cyclins), or loss of inhibitors (endogenous cdk inhibitors). Thus, cdks are suitable targets for cancer therapy. Many approaches are being designed to interfere with cdk activation. The most effective inhibition of cdk activity appears to come from rationally
designed small molecule inhibitors. Of these inhibitors, flavopiridol and UCN-01 are the most advanced in clinical development. This article discusses the mechanisms of action of flavopiridol and UCN-01 and the outcome of initial clinical trials with these compounds.

Causes of Cancer

Material & Methods

Cancer biomarkers can be DNA, mRNA, proteins, metabolites, or processes such as apoptosis, angiogenesis or proliferation. The markers are produced either by the tumor itself or by other tissues, in response to the presence of cancer or other associated conditions, such as inflammation. Such biomarkers can be found in a variety of fluids, tissues and cell lines. "A biological molecule found in blood, other body fluids, or tissues that is a sign of a normal or abnormal process, or of a condition or disease. A biomarker may be used to see how well the body responds to a treatment for a disease or condition. Also called molecular marker and signature molecule Diagnostic (screening) biomarker, Prognostic biomarker, Stratification (predictive) biomarker

Biomarkers play a key role in the diagnosis and management of patients with cancer, and are important for fulfilling the promise of precision medicine in oncology. However, although numerous biomarkers have been shown to have clinical validity, many have not undergone rigorous testing to demonstrate clinical utility so that they can be appropriately incorporated into clinical care. This review article highlights the characteristics of a good biomarker and the steps required to demonstrate clinical utility, and gives examples of both successful established biomarkers and
promising new tissue-based and circulating biomarkers on the horizon. Circulating tumour cell, circulating tumour DNA, clinical utility (Whiteman et al. 2016).

A Biomarker is the organic particle found in blood, other body liquids, or tissues that is an indication of a typical or anomalous process, or of a condition or disease. A biomarker might be utilized to perceive how well the body reacts to a treatment for a malady or condition. Likewise called molecular marker and mark particle. Cancer biomarkers are arranged by their diverse capacities: Biomarkers that Trigger Cells to Grow and Multiply Abnormally, Biomarkers That Support a Treatment's Cellular or Molecular Action, Biomarkers That Disrupt a Treatment's Cellular or Molecular Action, Detecting and Measuring Biomarkers to Develop a Personalized Anticancer Treatment Plan. Genomic biomarker, Transcriptomic biomarker, Metabolomics biomarker, Drug activity markers, Imaging biomarker.

**Breast cancer Biomarkers**

- Serum markers
  - CA 15.3
  - CA 27.29
  - CEA
- Hormone receptors
  - Estrogen receptors (ER)
  - Progesterone receptors (PR)
- Oncoproteins
  - Her-2/ neu
- Tumor supressor
  - p53
- Potential proteins (highly expressed)
  - MUC1
  - Mammaglobin
- Gene mutations
  - BRCA1
  - BRCA2
- Others
  - Adhesion molecules (E-selectin, ICAM-1, VCAM-1)
  - Cytokeratins
Flavopiridol

Flavopiridol (L86-8275 or HMR 1275) is a semi synthetic derivative of rohitukine, an alkaloid isolated from a plant indigenous to India.

Mechanisms of Action

Initially, flavopiridol was found to inhibit the epidermal growth factor receptor and protein kinase A (inhibitory concentration 50 % [IC\textsubscript{50}] = 21 and 122 μM, respectively). Flavopiridol was later shown to inhibit cell proliferation, at more physiologically relevant concentrations (IC\textsubscript{50} = 66 nM) when the drug was tested in the National Cancer Institute Developmental Therapeutics Program panel of 60 human tumor cell lines. The various mechanisms of action of flavopiridol are described below.

Cdk Inhibition

Studies using purified cdks showed that flavopiridol inhibits cdk1, cdk2, cdk4, and cdk6 (all IC\textsubscript{50} ~41 nM) as well as cdk7 (IC\textsubscript{50} = 300 nM) by competing with ATP. Analysis of the crystal structure of deschloro-flavopiridol bound to cdk2 showed that this flavopiridol congener, which has a phenyl ring instead of flavopiridol’s chlorophenyl, binds to the ATP-binding pocket of cdk2. Because cdks have a conserved structure, flavopiridol is expected to inhibit all cdks by docking in the ATP-binding site. In addition to binding to the ATP site of cdks, flavopiridol prevents the activation of most cdks due to inhibition of the cdk-activating kinase “CAK,” also known as cdk7, leading to the loss in phosphorylation at threonine 160/161, phosphorylation necessary for activation of most cdks, including cdk1, cdk2, cdk4, and cdk6. Flavopiridol also has been shown to inhibit cdk5, which is expressed in many cells but is only active in neuronal cells. Thus, flavopiridol may have therapeutic potential for Alzheimer’s disease, which is associated with increased cdk5 activation. Furthermore, flavopiridol inhibits cdk9, which together with T-type cyclins forms a complex known as positive transcription elongation factor b (P-TEFb), a kinase required for elongation control of RNA polymerase II. This binding does not appear to be competitive with ATP, suggesting that flavopiridol binds P-TEFb very tightly. Binding of flavopiridol to P-TEFb leads to inhibition of RNA polymerase II transcription.

Depletion of Cyclin D1

Exposure of MCF-7 breast cancer cells to flavopiridol resulted in a decrease in cyclin D1 protein within 3 hours, followed by a decrease in levels of cyclin D3 but not of cyclin D2 or cyclin E (IC\textsubscript{50} = 100-1000 nM). This depletion occurs at the mRNA level. Using luciferase reporter assays, we have shown that the depletion of cyclin D1 was preceded by a decline in cyclin D1 promoter activity leading to loss in cyclin D1 mRNA. Another study from our laboratory, using the nonmalignant breast epithelial cell line MCF10A, showed a G\textsubscript{1}/S cell-cycle arrest 12 hours after administration of flavopiridol, which was accompanied by a loss in cdk6 activity as measured by reduced Rb phosphorylation. Again, the loss in cdk6 activity was preceded by decline in cyclin D1. Cyclin D1 transcriptional repression is likely to be related to the inhibition of P-TEFb by flavopiridol (see “Inhibition of Transcription,” below).
Inhibition of Transcription

In addition to the effects of flavopiridol on cyclin D1 transcription, flavopiridol also modulates transcription in yeast, with clear changes in the families of genes involved in regulation of cell-cycle progression, phosphate and cellular energy metabolism, and guanosine 5’-triphospate (GTP)- and ATP-binding proteins [30]. These findings confirm that flavopiridol modulates transcription in several eukaryotic systems. To determine the exact mechanism by which flavopiridol modulates transcription, we studied the putative effects of flavopiridol on the activity of P-TEFb, a complex of cdk9 and T-type cyclins. P-TEFb phosphorylates the carboxyl-terminal domain of the large subunit of RNA polymerase II, thereby facilitating transcription elongation. Two recent reports have demonstrated that inhibition of P-TEFb by flavopiridol at a concentration of <100 nM, which is easily achieved in human clinical trials, results in blockage of RNA polymerase II transcription. Affected genes may include those involved in the regulation of apoptosis and cell cycle.

P-TEFb is required for activation of transcription of the HIV-1 genome by the viral transactivator Tat. Flavopiridol binding of P-TEFb was found to inhibit HIV replication at low concentrations (IC₅₀ = 8 nM), whereas flavopiridol concentrations of up to 100 nM did not inhibit cellular transcription. Thus, flavopiridol may have promising potential for AIDS therapy.

Angiogenesis Inhibition

Studies from our laboratory using human monocytes have demonstrated that flavopiridol prevents the vascular endothelial growth factor (VEGF) up regulation induced by hypoxia (IC₅₀ = 50-100 nM). Flavopiridol modulates VEGF through a decrease in VEGF mRNA stability. Studies from other laboratories also have shown an antiangiogenic effect of flavopiridol in various preclinical models: flavopiridol induced apoptosis in human umbilical vein endothelial cells and decreased blood vessel formation in the mouse Matrigel model of angiogenesis. It is not clear yet whether the antiangiogenic effect of flavopiridol is related to its cdk inhibitory effect.

Apoptosis Induction

Studies in our laboratory using head and neck squamous cell carcinoma (HNSCC) cell lines have shown that flavopiridol induces apoptosis as evidenced by the increase in sub-G₁ DNA content (IC₅₀ = 100-1,000 nM). Flavopiridol even induced apoptosis in HN30, a HNSCC cell line that is resistant to apoptosis induction by DNA-damaging agents such as bleomycin and γ-irradiation. Flavopiridol treatment (i.p., daily for 5 days) induced apoptosis in the HNSCC xenograft HN12 as detected by terminal deoxynucleotidyltransferase-mediated dUTP-biotin end labeling (or TUNEL), with significant reduction in tumor size. Furthermore, flavopiridol resulted in depleted cyclin D1 levels in the HN12 tumor xenograft, whereas levels of cyclin D3 and cyclin E remained constant. The mechanism(s) for apoptosis induction by flavopiridol are still under investigation. It is unclear whether the cdk-inhibiting activity of flavopiridol is required for induction of apoptosis. A recent report showed that flavopiridol inhibits transcription of genes that encode apoptosis regulators. Further studies into the mechanism of apoptosis by this agent are warranted.
Induction of Differentiation

Flavopiridol was shown to induce mucinous differentiation in lung carcinoma cells accompanied by loss in cdk2 activity. Again, it is unclear whether the induction of differentiation is related to the cdk-inhibitory properties of flavopiridol.

Clinical Studies

The first clinical trial using flavopiridol was conducted at the National Cancer Institute (NCI). The promising results of this trial prompted the initiation of many clinical trials testing flavopiridol with different schedules, as well as in combination with standard chemotherapeutic agents.

Seventy-Two-Hour Continuous Infusion Studies

In the first trial of flavopiridol at NCI, 76 patients received a 72-hour continuous infusion of flavopiridol every 2 weeks. This initial schedule was chosen based on the “cytostatic effects” observed in preclinical models with prostate and other solid tumor models. Thus, using this schedule, tumor regressions in patients were not likely. However, significant stability was the expected outcome with this schedule. The maximum tolerated dose (MTD) was 50 mg/m²/day, with a dose-limiting toxicity of secretory diarrhea. To understand this side effect, mechanistic in vitro studies were conducted that found that flavopiridol modifies chloride secretion by intestinal epithelial cells. Using antidiarrheal prophylaxis, the MTD could be increased to 78 mg/m²/day, which resulted in a dose-limiting toxicity of symptomatic hypotension and proinflammatory syndrome. This toxicity was reversible and its etiology is under study. A patient with refractory renal cancer exhibited a partial response (tumor shrinkage >50 %), while minor responses (tumor shrinkage <50 %) were observed in one patient with non-Hodgkin’s lymphoma, one patient with colon cancer, and one patient with renal cancer. Cdk-inhibitory plasma concentrations of flavopiridol (300-500 nM) were achieved.

The 72-hour continuous infusion of flavopiridol 50 mg/m²/day every 2 weeks has been used in other phase I/II clinical trials. In addition to diarrhea and fatigue, a few patients exhibited arterial and venous thromboses following flavopiridol treatment. A complete response was observed in a patient with refractory metastatic gastric cancer. In contrast, in a phase II trial conducted in 14 patients with metastatic gastric cancer, one minor response was observed while histology and radiography showed tumor necrosis in several patients. There were two partial responses among 35 patients with metastatic renal cancer. In this trial, pharmacokinetic analysis demonstrated that the systemic glucuronidation of flavopiridol is inversely associated with the risk of developing diarrhea. In another trial, 20 patients with metastatic non-small cell lung cancer (NSCLC) were treated with 72 hour infusional flavopiridol (50 mg/m²/day). Although no objective responses were observed, six patients exhibited significant disease stabilization (≥3 months) with an overall survival of ~7 months. Preliminary results from a phase II trial in patients with metastatic colorectal cancer showed no objective responses among 10 evaluable patients. Flavopiridol plasma concentrations in these trials, except for two in which no drug plasma concentrations were published, were in the range of those reported for the NCI trial.
One-Hour Infusion Studies

In order to obtain a higher therapeutic index of flavopiridol, we administered flavopiridol to leukemia/lymphoma and HNSCC xenografts as a bolus for 5 consecutive days. A strong induction of apoptosis and antitumor effects was observed in these models. Therefore, we initiated another phase I trial in which patients received flavopiridol as a 1-hour infusion for 5 consecutive days every 3 weeks. The MTD was 37.5 mg/m²/day and dose-limiting toxicities were neutropenia, fatigue, and diarrhea. The five daily 1-hour infusions every 3 weeks resulted in a flavopiridol concentration of ~1.5 μM. To reach higher drug plasma concentrations, flavopiridol was administered as 1-hour infusions at a higher dose per day (50 mg/m²/day) for 3 consecutive days every 3 weeks. This treatment resulted in a flavopiridol plasma concentration of ~4 μM and associated toxicities of neutropenia, vomiting, diarrhea, and pro-inflammatory syndrome. Although no objective responses were observed, this treatment stabilized disease in 3 (one mantle-cell lymphoma, one NSCLC, one melanoma) of 12 patients.

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Following DNA damage, the G₂ checkpoint is activated, which allows the cell to remain in G₂ until all DNA damage is repaired, allowing cells to enter M phase with “intact” DNA (Fig. 1). However, UCN-01 treatment of DNA-damaged cells abrogates the G₂ checkpoint (IC₅₀ ~50 nM), which allows the cells to progress into M prior to completion of DNA repair, leading to apoptosis (Fig. 1). The UCN-01 G₂ checkpoint abrogation was found to involve Cdc25C, the cdk1 (Cdc2)-activating phosphatase. Recent studies have shown that UCN-01 inhibits phosphorylation of Cdc25C by the
kinase chk1 (Fig. 1). The ability of UCN-01 to induce apoptosis in response to DNA damage is being explored in combination trials with standard chemotherapeutic agents.

UCN-01 has also been shown to abrogate the S phase checkpoint, which is activated upon DNA damage. The target of UCN-01 is likely to be chk1, which recently was shown to be involved in the S-phase checkpoint

**Induction of Apoptosis**

Our laboratory and others have demonstrated apoptosis induction by UCN-01, particularly in a panel of HNSCC cell lines. Furthermore, UCN-01 has potent antitumor effects on HN12 tumor xenograft after treatment for 5 days (A. Senderowicz, submitted for publication). This antitumor effect was associated with depletion of cyclin D3 and an increase in p27Kip1 and p21waf1. Although the mechanism of UCN-01-induced apoptosis (IC50 = 100-1,000 nM) is still unknown, several reports demonstrate that, in some in vitro models, UCN-01 can down regulate some anti-apoptotic proteins, similar to flavopiridol.

**Clinical Studies**

The first clinical trial with UCN-01 was recently completed at NCI. The initial schedule was a 72-hour continuous infusion every 2 weeks. However, in the first nine patients who received infusional UCN-01, the half-life appeared to be 100-fold longer than that observed in preclinical models. Furthermore, the initial concentrations achieved in plasma were ~4 to 7 μM. This again differed greatly from the findings in animal models, in which drug plasma concentrations of ≥1 μM were universally lethal. It appeared that UCN-01 in humans strongly binds to plasma α1-acidic glycoprotein. Therefore, treatment schedules were successfully changed to 36-hour continuous infusion in patients receiving ≥12 mg/m2/day every 4 weeks. Dose-limiting toxicities were nausea/vomiting, symptomatic hyperglycemia, and pulmonary toxicity. The mean half-life was approximately 588 hours and the total drug plasma concentration ranged from 30 to 40 μM. The concentration of “free” salivary UCN-01 concentrations at MTD was ~100 nM, which has been shown to modulate cell-cycle processes in vitro. Similar results were obtained in plasma samples after ultracentrifugation. A partial response was observed in a patient with melanoma. A complete response was observed in a patient with refractory alk-positive anaplastic large-cell lymphoma. This patient’s treatment was discontinued ≥1 year ago and he is still disease-free after 4 years of therapy.

Plasma samples from patients who received UCN-01 were shown to induce a 40 % to 70 % abrogation in an ex vivo G2 checkpoint assay, reflecting free plasma UCN-01. Furthermore, target modulation by UCN-01 was demonstrated by the level of phosphorylation of the PKC substrate adducin. Adducin phosphorylation in bone marrow and tumor samples taken during UCN-01 treatment was significantly reduced compared with pretreatment samples.

Currently, phase I clinical trials are exploring novel schedules of UCN-01 (1- to 3-hour infusion every 4 weeks). Furthermore, phase I clinical trials using a combination of cytotoxic agents (cisplatin, 5-fluorouracil, fludarabine) with UCN-01 are ongoing.
Results


The ubiquitin-proteasome pathway is a major pathway for the targeted degradation of proteins and involves multistep enzymatic reactions catalyzed by a cascade of enzymes, including ubiquitin-activating enzyme E1, ubiquitin-conjugating enzyme E2, and ubiquitin ligase E3. Ubiquitin is first activated by binding to E1 through a thioester bond between a cysteine residue at the active site of E1 and the C-terminus glycine (G76) of ubiquitin. Activated ubiquitin in an E1-ubiquitin complex is then transferred to E2, which also forms a thioester bond between its active-site cysteine residue and the G76 of ubiquitin. Finally, ubiquitin is covalently attached to the target protein through an isopeptide bond between the G76 of ubiquitin and the ε amino group of an internal lysine residue of the target protein, catalyzed by E3 ubiquitin ligase. Through multiple runs of reactions, ubiquitin is covalently attached to substrates to form K48-linked polyubiquitin conjugates that are rapidly recognized and degraded by the 26S proteasome. Recent data have shown that proteins can also be monoubiquitinated or polyubiquitinated through K63 linkage, leading to altered protein activity and subcellular localization, rather than degradation. A diagram of ubiquitination reaction and the three potential fates of proteins after ubiquitination are illustrated.

E3 ubiquitin ligase is an enzyme that binds to specific protein substrates and promotes the transfer of ubiquitin from a thiolester intermediate to amide linkages with proteins or polyubiquitin chains. Because they serve as the specific substrate-recognition element of the system, E3 ligases play an important role in ubiquitin-mediated proteolytic cascade. There are approximately 1000 E3 ligases in the human genome that can be classified into three major types,
based on their domain structure and substrate recognition. The first class comprises N-end rule ubiquitin ligases that target protein substrates bearing specific destabilizing N-terminal residues, including Arg, Lys, His (type I), and Phe, Trp, Leu, Tyr, and Ile (type II). One recent example of protein degradation by the Ub-dependent N-end rule pathway is Drosophila inhibitor of apoptosis protein (IAP). The second type of E3 is HECT, with the first family member being E6-associated protein (E6-AP), which, together with oncoprotein E6, promotes p53 ubiquitination and degradation. HECT E3 ligases contain an approximately 350-amino acid C-terminal region homologous to that of E6-AP, with a conserved active-site cysteine residue near the C-terminus, through which HECT domain E3 ligases form thioester intermediates with Ub. N-terminal regions are highly variable and may be involved in substrate recognition. The third and largest type of E3 ligase is the Really Interesting New Gene (RING) family, which contains a classic C_3H_2C_3 or C_3HC_4RING finger domain with a characteristic linear sequence of Cys-X_2-Cys-X_9-39-Cys-X_1-3-His-X_2-3-Cys/His-X_2-Cys-X_4-48-Cys-X_2-Cys, where X can be any amino acid. A RING finger domain binds to two zinc atoms per molecule in a cross-braced system, where the first and third pairs of cysteine/histidine form the first binding site and where the second and fourth pairs of cysteine/histidine form the other.

E3 ubiquitin ligases exist and act as a single peptide [such as murine double minute 2 (Mdm2) and X-linked IAP (XIAP)] or as multiple component complexes [such as Skp1-Cullin-F-box protein (SCF)]. Through the covalent modification of a vast repertoire of cellular proteins with ubiquitin, E3 ubiquitin ligases regulate almost all aspects of eukaryotic cellular functions and biologic processes. Accumulating data have strongly suggested that deregulation of E3 ligases contributes to cancer development and that overexpression of E3 ligases is often associated with poor prognosis. Thus, E3 ligases, which determine the specificity of protein substrates and are themselves “drugable” enzymes, can serve as potential cancer targets as well as cancer biomarkers.

E3 Ubiquitin Ligases as Potential Cancer Targets

An ideal cancer target meets the following criteria: 1) it plays an essential role in cancer genesis, and/or is required for the maintenance of cancer cell phenotype, and/or is apoptosis-protective and confers cancer cells resistance to apoptosis; 2) it is overexpressed in cancer cells, and its overexpression is associated with a poor prognosis of patient survival; 3) inhibition of its expression or activity induces growth suppression and/or apoptosis in cancer cells; 4) it is “drugable,” meaning that it is an enzyme (e.g., kinase) or a cell surface molecule (e.g., membrane-bound receptor) that can be easily screened for small-molecule inhibitors or that can be targeted by a specific antibody; and 5) most importantly, it is not expressed or is expressed at a very low level in normal cells, and its inhibition has a minimal effect on normal cell growth and function. Thus, inhibition of such a target would achieve a maximal therapeutic index with minimal toxicity. The E3 ubiquitin ligases discussed below would meet some of these criteria.

Mdm2 E3 Ubiquitin Ligase and p53

Mdm2 encodes a 90-kDa protein that was first identified as the gene responsible for the spontaneous transformation of an immortalized murine cell line BALB/c 3T3. It contains a p53-binding domain at the N-terminus and a RING domain at the C-terminus. The p53-binding domain of Mdm2 or Hdm2 (human counterpart of Mdm2) binds to the tumor
suppressor p53, whereas the RING domain acts as an E3 ubiquitin ligase to promote rapid degradation of p53. Both in vitro and in vivo studies indicated that oncogeneic activity of Mdm2 is mainly attributable to its binding and degradation of p53. P53 is a classic tumor suppressor that is inactivated in more than 50% of human cancers. Under unstressed conditions, the p53 level is very low in cells due to Mdm2 binding and degradation. On DNA damage by ionizing radiation or anticancer drugs, p53-Mdm2 binding is dissociated as a result of p53 phosphorylation and acetylation, leading to p53 activation. Activated p53 acts as a transcription factor that Trans activates a number of genes, leading to growth arrest (to repair damaged DNA) or apoptosis (if damage is too severe to repair).

Ubiquitin pathways in the regulation of protein degradation and function Ubiquitin is first attached to E1 ubiquitin-activating enzyme in the presence of ATP. The activated ubiquitin is then transferred to E2 ubiquitin-conjugating enzyme. E3 ubiquitin ligase recognizes a protein substrate, recruits an E2-ubiquitin complex, and catalyzes ubiquitin transfer from E2 to substrate. A single run of the reaction causes monoubiquitination of a target protein that could change its function, whereas multiple runs of the reaction lead to polyubiquitination of the substrate. Depending on ubiquitin-ubiquitin linkage, polyubiquitinated proteins can either be activated (through K63 linkage), or recognized and degraded by the 26S proteasome (through K48 linkage).

Hdm2 E3 ligase inhibitor

A family of small molecules—HLI98 series—was identified through an HTS of a chemical library of 10,000 compounds using an in vitro Hdm2 autoubiquitination assay. Follow-up experiment showed that the compound indeed inhibited Hdm2 activity, as well as other E3 ligases and even E2 ligases at higher concentrations. In cell-based assays, the compound stabilized p53 and Mdm2, and activated p53-dependent transcription and apoptosis, but also had p53-independent cytotoxicity. Furthermore, as expected, the compound worked much better in cancer cells containing wild-type p53 than in those containing mutant p53 because targeting Hdm2 should, in theory, have little or no effect on human cancers with mutant p53. However, in vivo antitumor activity of HLI98, using human xenograft models, has not been reported. Nevertheless, this proof-of-concept study indicated that Hdm2 E3 is a valid cancer target and that it is
possible to identify more potent inhibitors of Hdm2 E3 ligase as a novel class of anticancer drugs for future discovery and development.

**Compounds disrupting Hdm2-p53 binding**

Three classes of structurally distinctive compounds, namely, Nutlin, RITA (Reactivation of p53 and Induction of Tumor cell Apoptosis), and a nonpeptide Mdm2 inhibitor (MI-17), were reported to disrupt Hdm2-p53 binding. Historically, it has been difficult to develop small-molecule inhibitors to disrupt large protein-protein interactions. However, the crystal structure of Mdm2-p53 peptide binding revealed that binding relies on the contact of the p53 peptide side chains of Phe19, Trp23, and Leu26 with the N-terminus of Mdm2 (amino acids 17–125) in a deep hydrophobic pocket, which made it possible for small molecules to disrupt binding. Indeed, the Nutlin series was identified through a screening of a diverse library that disrupted Mdm2-p53 peptide binding, whereas structure-based design on Mdm2-p53 binding pocket led to identification of a potent nonpeptide Mdm2 inhibitor MI-17 with a chemical structure different from that of Nutlin. Conversely, RITA was identified through a cell proliferation assay using a pair of isogenic cancer cell lines differing in p53 status. RITA bound to p53 and prevented p53-Hdm2 interaction. Compared to Hdm2 E3 ligase inhibitors, Mdm2-p53 binding inhibitors appeared to be much more potent and specific in activating the p53 pathway, leading to growth arrest, apoptosis, and *in vivo* tumor growth inhibition. Again, mechanistically, these compounds will only work in human cancers harboring wild-type p53 and, preferentially, with Mdm2 overexpression. This certainly turned out to be the case.

Two major concerns are associated with these compounds, which induce p53 accumulation through Mdm2 manipulation. The first concern is the therapeutic window or the selectivity between normal and cancer cells. Although it is still unclear mechanistically, the activation of p53 in normal cells by these compounds mainly caused growth arrest, rather than apoptosis, making it possible to achieve a therapeutic window by adjusting the dose regime and the duration of the treatment (S. Wang, personal communication). The second concern is the oncogenic activity of Mdm2 independent of p53. Because Mdm2 itself is a p53 target, p53 activation, as a result of either approach, would cause significant accumulation of Mdm2. An increased amount of even ligase-deficient Hdm2 might actually promote tumor growth. This potential side effect needs to be further addressed. Finally, in addition to Mdm2, two additional RING proteins, COP1 and PIRH2, were determined to be p53 targets and to promote p53 ubiquitination and degradation. Indeed, COP1 and PIRH2 were overexpressed in a subset of human cancers with increased p53 ubiquitination. Further validation of COP1 and PIRH2 as promising cancer targets is a prerequisite to initiating a library screen for their specific inhibitors.

**IAP and Caspases**

The IAP family has at least eight members, including XIAP, cIAP-1, cIAP-2, Ts-IAP, NAIP, survivin, Livin/ML-IAP, and Apollon/Bruce. They all contain one or several baculoviral IAP repeat (BIR) domains that are required for the suppression of apoptosis. Some family members also have a RING finger domain at the C-terminus for the ubiquitination and degradation of caspases. In XIAP, BIR3 (the third BIR domain) potently inhibits the activity of the
active caspase-9, whereas the linker region between BIR1 and BIR2, as well as the BIR2 domain itself, selectively targets active caspase-3 or caspase-7. Thus, IAP suppresses apoptosis by binding to and by inhibiting active caspase-3, caspase-7, and caspase-9 through BIR domains. In apoptotic cells, caspase inhibition by IAP is negatively regulated by a mitochondrial protein, second mitochondria-derived activator of caspase (Smac). Smac physically interacts with multiple IAPs and relieves their inhibitory effect on caspase-3, caspase-7, and caspase-9. Smac binds to the BIR3 domain of XIAP through four N-terminal residues (AVPI) that recognize a surface groove on BIR3. These four amino acids are conserved in three Drosophila proteins (Reaper, Grim, and Hid) that induce apoptosis by eliminating the binding of Drosophila IAP to caspases.

IAP as a promising cancer target has been extensively validated by over expression, silencing, or the use of a Smac-derived AVPI peptide that binds to IAPs to free up caspases. Indeed, over expression of IAP suppressed apoptosis induced by a variety of stimuli, whereas down regulation of XIAP or survivin through antisense RNA or siRNA has been shown to induce apoptosis in many human cancer cell lines. Furthermore, Smac peptide consisting of AVPI sequence sensitized many human cancer cells to apoptosis induced by conventional cancer therapies both in vitro and in vivo, indicating that it is feasible to identify AVPI-like small molecules to disrupt IAP-caspase binding. The current effort, therefore, was focused on IAP-caspase binding inhibitors. Analogous to the Mdm2-p53 case, the use of small molecules to disrupt protein-protein binding was made possible by a well-defined small binding packet between the IAP BIR3 domain and the AVPI peptide of Smac. At least five classes of such compounds have been discovered so far, and their structures have been summarized in a recent review article.

The first class comprises tripeptides of unnatural amino acids that were developed through a structure-based design targeting the BIR3 domain of XIAP. The compounds induced apoptosis in a number of human cancer cell lines by releasing active caspase-9 from XIAP binding. The structure-based computational screening of a three-dimensional structure database of traditional herbal medicines led to the discovery of embelin as a potent inhibitor of XIAP-caspase-9 binding. Embelin activated caspase-9, inhibited cell growth, and induced apoptosis in prostate cancer cells with high levels of XIAP, with minimal effect on normal prostate epithelial and fibroblast cells, containing low levels of XIAP expression.

The next two classes comprise compounds targeting BIR2 or BIR2 link regions to disrupt binding to caspase-3. Aryl sulfonamide, identified through the biochemical screening of a combinatorial chemical library, disrupted XIAP-caspase-3 interaction and sensitized cancer cells to the activator of the death receptor pathway. A polyphenylureas series was identified with chemical library screening using an enzyme depression assay by overcoming XIAP-mediated suppression of caspase-3. These series of compounds indeed increased caspase activity had broad activity against cancer cell growth as tested in 60 NCI cancer cell lines, sensitized cancer cells to chemotherapeutic drugs, and inhibited tumor cell growth in xenograft models in mice with limited toxicity to normal tissues. This class of compounds has been shown recently to activate caspase-3 and caspase-7 and to directly induce the apoptosis of leukemia cell lines and primary samples from acute myelogenous leukemia patients without much lethal effect on normal hematopoietic cells.
Finally, a Smac mimic small-molecule compound was identified through structure-based design using computer-simulated conformations of AVPF as a guide. The compound bound to XIAP, c-IAP1, and c-IAP2 to activate caspase-3 and sensitized cancer cells to apoptosis induced by TNF [tumor necrosis factor]-related apoptosis-inducing ligand (TRAIL) and tumor necrosis factor α. We further evaluate the compound in three breast cell lines with various levels of IAP. Acting alone, Smac mimic compound was quite potent with a cytotoxic IC$_{50}$ of 3.8 nM in high IAPs expressing MDA-MB-231 cells, but was inactive at a much higher concentration in low IAPs expressing T47D and MDA-MB-453 cells. In fact, as low as 2.5 nM Smac mimic alone was sufficient to activate caspase-3 and to induce apoptosis in MDA-MB-231 cells. In combination treatments with TRAIL or etoposide, Smac mimic significantly sensitized cells to growth suppression and apoptosis in MDA-MB-231 cells, but too much lesser extent in T47D and MDA-MB-453 cells. Thus, in these cell lines, Smac mimic compound acts in an apparent IAP-dependent manner to induce apoptosis alone, as well as to sensitize breast cancer cells to TRAIL- or etoposide-induced apoptosis through caspase-3 activation.

![Extrinsic Pathway and Intrinsic Pathway](image)

Targeting IAPs for caspase activation and apoptosis induction IAP binds to caspases through its BIR2 or BIR3 domain and promotes the ubiquitination and degradation of caspases (Casps) through its C-terminal RING domain. Small-molecule inhibitors that disrupt IAP-caspase binding (AVPI-like) or yet-to-be discovered IAP E3 ligase inhibitor (E3I) would either release caspases or induce accumulation of caspases, leading to apoptosis induction.

**SCF E3 Ubiquitin Ligases and Their Substrates**

SCF and SCF-like complexes comprise the largest family of E3 ubiquitin ligases that consist of Skp1, Cullins, F-box protein, and ROC/Rbx/SAG (Sensitive to Apoptosis Gene) RING finger protein. The crystal structure of the SCF-Rbx complex revealed that Cul-1 acts as a scaffold that binds Skp1-F-box$^{Skp2}$ (the protein substrate-recognition complex; at its N-terminus) and Rbx1 (which recruits E2; at its C-terminus). Thus, SCF E3 ubiquitin ligases may serve as scaffolds that position substrates and E2 enzyme optimally for ubiquitin transfer. Through various F-box proteins acting as
substrate receptors, SCF ligases recognize many protein substrates and promote their ubiquitination and degradation, thus regulating a variety of biologic processes. For example, through Skp2, which binds to cyclin-dependent kinase inhibitors p27, p21, and p57, SCF E3 promotes their ubiquitination and degradation, thus promoting G1→S progression. Skp2 also binds to c-Myc to promote its ubiquitination and degradation and, at the same time, acts as a coactivator to enhance c-Myc-induced S-phase transition and to activate c-Myc target genes. Through the F-box protein β-TrCP, SCF E3 ligase promotes the ubiquitination and degradation of Emi-1 (early mitotic inhibitor), an inhibitor of the anaphase-promoting complex, to control meiotic and mitotic progression. β-TrCP also binds to IκB and β-catenin and, together with other components of SCF ligase, promotes their ubiquitination and degradation, thus regulating NFκB and Wnt signaling pathways. Accumulated evidence strongly suggested that abnormal regulations of SCF E3 ubiquitin ligase contribute to uncontrolled proliferation, genomic instability, and cancer.

The validation of whether SCF E3 ubiquitin ligase is an appealing cancer target has been mainly focused on its components, particularly Skp2, β-TrCP, and SAG, using either over expression or silencing (through antisense or siRNA) approaches. Skp2 over expression in gastric carcinoma cells decreased the level of p27, increased cell growth rate, rendered cancer cells more resistant to actinomycin D-induced apoptosis, and increased their invasion potential. Tissue-specific expression of Skp2 in the prostate gland of a mouse transgenic model caused significant downregulation of p27 level and marked tissue over proliferation, leading to hyperplasia, dysplasia, and low-grade carcinoma. Skp2, when targeted for expression in T-lymphoid lineage, cooperated with activated N-Ras to induce T-cell lymphomas with a short latent period and high penetrance, indicating that Skp2, as a protooncogene, is involved in the pathogenesis of lymphomas. Conversely, down regulation of Skp2 using an antisense oligonucleotide remarkably suppresses the growth of small cell lung cancer cells. siRNA silencing of Skp2 has been shown to inhibit the growth of melanoma cells, oral cancer cells, glioblastoma cells, and lung cancer cells. Similarly, over expression of β-TrCP increased NFκB activity and chemoresistance, whereas silencing of β-TrCP by siRNA reduced NFκB activation and chemoresistance in pancreatic cancer cells. Transgenic mice with β-TrCP1-targeted expression in the intestine, liver, and kidney had an increased incidence of tumor formation in these organs. Conversely, silencing β-TrCP1 through siRNA or over expression of dominant-negative mutant was shown to suppress the growth and survival of human breast cancer cells. In the case of SAG, the second member of ROC/Rbx family and a RING component of SCF, its over expression protected cells and tissues from apoptosis induced by redox reagents and by ischemia/reperfusion-generated hypoxia in a RING domain-dependent manner. SAG over expression also promotes cell growth under serum-starved conditions, whereas antisense SAG transfection inhibits tumor cell growth.

Although substantial progress has been made in this area, SCF E3 ligase as a cancer target suffers from several intrinsic drawbacks. The first is specificity. The same SCF E3 ligase can promote the degradation of either oncogenes or tumor-suppressor genes, dependent on different F-box proteins or even the same F-box protein. The therapeutic outcome of ligase inhibitors has to be cell context-dependent, which is hard to manage in cancer patients without a thorough understanding of the mechanism. Secondly, assay complexity is a big issue, although several screening assays for E3 ligase inhibitors have been developed recently. SCF is a multiple component E3 ligase, and its intrinsic enzymatic mechanism is still unclear, except that the core ligase components Rbx1/ROC1-Cullins have been shown to promote
autoubiquitination in an *in vitro* assay. Indeed, it is feasible to identify general inhibitors against Cul-ROC/SAG core E3 ligase using an *in vitro* assay described for the inhibitor screening of APC2/APC11 core ligase; such inhibitors would not, however, have a desired specificity against any particular SCF complex. It is uncertain to conduct high-throughput screening (HTS) using all SCF E3 components because, unlike kinases or proteases, SCF ligase does not contain an evident central enzymatic active site to which small molecules could bind. A three-dimensional structure-based computer design strategy has been proposed to assess whether interfaces among SCF components are suitable for small-molecule binding. An alternative approach is to screen for inhibitors that disrupt binding between SCF components. One example is the development of an HTS assay for inhibitors of Cks1-Skp2 interaction that would lead to p27 accumulation. Figure 4 illustrates two potential approaches to target SCF-Skp2 E3 ligase with the expected outcome of p27 accumulation and growth inhibition. Nevertheless, it appears that SCF E3 ligase itself may not be a practical target per se. However, its components may serve as cancer biomarkers for further development and use in cancer clinics.

![Diagram](image.jpg)

Targeting SCF-Skp2 for p27 accumulation and growth inhibition SCFE3 ubiquitin ligase consists of four components: scaffold protein Cullins to link Skp1 and Rbx/SAG; adaptor protein Skp1 to link Cullins and F-box protein; RING protein Rbx/ROC/SAG to recruit E2; and F-box protein to recognize substrate. In the SCF-Skp2 E3 ligase that promotes the ubiquitination and degradation of p27, a small protein Cks1 is also involved. Small molecules that can either inhibit core E3 ligase activity (E3I) or disrupt Cks1/Skp2/p27 binding would induce p27 accumulation and cause growth arrest.

**Ligase and Their Components as Cancer Biomarkers**

Early diagnosis and treatment of cancer would significantly improve the survival of cancer patients. The development of cancer biomarkers for early detection or prognosis prediction is of significant importance. An ideal cancer biomarker will meet some criteria for a cancer target (e.g., high expression in cancer tissues, but not in normal tissues, with a
causal relationship with cancer genesis, development, or metastasis). In addition, it should be a secretory protein that is readily obtained and identified from a patient's body fluids, such as serum, urea, stool, and sputum. For intracellular biomarkers to which E3 ubiquitin ligases belong, it should be over expressed or it should have a high frequent mutation rate so that it can be readily identified by immune his to chemical or mutational analyses using tumor tissues from biopsy. Traditional biomarkers of cell proliferation, such as Ki-67 and PCNA, have had a mixed clinical track record. New and more reliable cancer markers are being searched and developed. Due to the over expression of some E3 ubiquitin ligases in a number of human cancers with associated poor prognosis, it is possible that these E3 ligases (such as Hdm2 and the F-box protein Skp2) can be further characterized and developed as useful cancer biomarkers.

**Hdm2**

Although it is normally expressed at a low level, Hdm2 is over expressed through gene amplification, increased transcription, or enhanced translation in a variety of human cancers, including breast carcinomas, soft tissue sarcomas, esophageal carcinomas, lung carcinomas, glioblastomas, and malignant melanomas. Among the 28 human tumor types examined, the overall frequency of Hdm2 gene amplification is 7 %, with the highest frequency being observed in soft tissue tumors (20 %). Furthermore, high Hdm2 levels are often associated with poor prognosis, with an increased likelihood of distant metastases and with a poor response to therapeutic drugs. Due to the important role of Hdm2 in promoting the degradation of tumor suppressor p53, the development of Hdm2 as a cancer biomarker, particularly in soft tissue sarcomas, is highly desirable.

**IAPs**

The expression and prognostic significance of RING-containing IAPs, such as XIAP, c-IAP1, and c-IAP2, have been extensively studied in human tumor and cells lines with mixed results. The study with 60 NCI cancer cell lines revealed that higher levels of XIAP or c-IAP1 proteins correlated with sensitivity or resistance to some chemotherapeutic drugs, respectively. Conversely, acute myeloid leukemia (AML) patients with lower levels of XIAP proteins had a significantly longer survival, with a tendency toward a remission longer than that of patients with higher levels of XIAP. Similarly, high expression levels of XIAP correlated with poor overall survival in childhood de novo AML. In another study, however, expression levels of XIAP had no prognostic impact on AML patients. In radically resected non-small cell lung cancer patients, a high XIAP predicted a longer overall survival. In cervical carcinoma, the basal expression levels of IAPs had no prognostic significance. In clear cell renal carcinomas, a significant inverse correlation was achieved between XIAP expression and tumor aggressiveness, and patients' survival. Furthermore, survivin, a RING-less IAP, was found to be over expressed in most common human cancers, but not in normal terminally differentiated adult tissues. The resistance of cancer cells to conventional cancer therapy and a worse clinical prognosis are usually correlated with a high expression of survivin. Due to these mixed results, it is unlikely that IAPs, with the probable exception of survivin, will be developed as useful cancer biomarkers.
SCF Components: Skp2, β-TrCP, Cul4A, and SAG

It has been well documented that Skp2 acts as an oncogene mainly by targeting p27 for ubiquitination and degradation. Overwhelming evidence showed that Skp2 is over expressed in almost all major human cancers, including carcinomas of the breast, colon, lung, brain, prostate, and liver, among many other human cancers. In most cases, Skp2 overexpression is inversely correlated with p27 expression and is directly correlated with poor clinic prognosis (for a review, see Nakayama and Nakayama. Thus, Skp2-p27 inverse correlation may deserve further characterization for clinical use as a prognostic index.

Among other SCF components, expression of β-TrCP1 was found to be elevated in colon cancers (particularly in those with metastases), pancreatic carcinomas, and hepatoblastomas. Over expression of β-TrCP2 was also detected in primary prostate, breast, and gastric cancers, as well as in the cell lines derived from these cancers. Cul4A was recently found to be involved in Mdm2-mediated p53 degradation, as well as in DDB1-Skp2-mediated p27 degradation. Cul4A gene was amplified and over expressed in primary breast cancers and hepatocellular carcinomas. Finally, SAG was over expressed in a subset of colorectal carcinomas and in non-small cell lung cancers. Importantly, high SAG expression was correlated with poor patient survival and could serve as a useful prognostic marker.

Conclusion

A Cancer marker or tumor marker is a biomarker found in blood, pee, or body tissues that can be raised by the proximity of at least one sorts of development. There are different tumor markers, each illustrative of a particular alignment. In addition to their use in cancer medicine, biomarkers are often used throughout the cancer drug discovery process. For instance, in the 1960s, researchers discovered the majority of patients with chronic myelogenous leukemia possessed a particular genetic abnormality.

A biological molecule found in blood, other body fluids, or tissues that is a sign of a normal or abnormal process, or of a condition or disease. A biomarker may be used to see how well the body responds to a treatment for a disease or condition. NIH Biomarkers Definitions Working Group: "A characteristic that is objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes, or pharmacologic responses to a therapeutic intervention." World Health Organization: "Any substance, structure, or process that. Biomarkers are characteristics of the body that you can measure. So your blood pressure is actually a biomarker. Biomarkers are very important to medicine in general. We're all used to going to the doctor and getting all our test results, right, and even imaging x-ray results or CAT scans.

Most human malignancies display aberrations in the Rb pathway due to cdkhyperactivation. Thus, cdk5 may be an excellent therapeutic target. The cdk inhibitors flavopiridol and UCN-01 are the first small molecules that target cdk5. Their therapeutic value in cancer patients currently is being evaluated in phase I/II clinical trials. In addition, several novel compounds that target molecular pathways involved in cancer progression are under development. Extensive testing of effective novel compounds, including flavopiridol and UCN-01, in phase II clinical trials is needed to assess
appropriate treatment schedules as well as the possibility of their use in combination with conventional cytotoxic agents.

All these studies showed convincingly that IAPs are valid cancer targets and that disrupting their binding to caspases would release active caspases to induce apoptosis preferentially in IAPs over expressing human cancer cells, with less toxicity on normal cells having low IAP expression. Conversely, IAPs have been shown to act as ubiquitin ligases to promote the ubiquitination and degradation of caspase-3, caspase-9, and Smac; mutations of the RING domain, which are required for E3 ligase activity, reduced the apoptotic activity of XIAP. Thus, targeting their ubiquitin ligases appears to be a feasible approach to increasing the levels of caspases and Smac, thus inducing apoptosis in cancer cells or sensitizing cancer cells to conventional cancer therapies. It is of concern that even ligase activity is inhibited; however, IAPs may still bind to caspases and prevent caspase activation and apoptosis induction. Thus, specific inhibitors of IAP E3 ligase, which are yet to be discovered, would be more effective in combination therapy with chemotherapeutic drugs or IAP-caspase binding inhibitors. Figure 3 illustrates IAP targeting to activate caspases and to induce apoptosis.

E3 ubiquitin ligases regulate a variety of biologic processes, including cell growth and apoptosis, through the timely ubiquitination and degradation of many cell cycle- and apoptosis-regulatory proteins. Abnormal regulation of E3 ligases has been convincingly shown to contribute to cancer development. Thus, targeting E3 ubiquitin ligases for cancer therapy has gained increasing attention, which is further stimulated by the recent approval of a general proteasomal inhibitor, bortezomib (Velcade, Millennium, MA), for the treatment of relapsed and refractory multiple myeloma, as well as for the discovery of a new class of proteasome inhibitors. In contrast to general proteasome inhibitors, targeting a specific E3 would selectively stabilize a specific cellular protein regulated by a particular E3, thus avoiding some unwanted effects on other cellular proteins. This would, therefore, achieve a high level of specificity with less (at least in theory) associated toxicity. Because several HTS assays are now in place for the rapid screening of small molecular inhibitors of E3 ligases, it is anticipated that, in the near future, specific inhibitors of E3 ubiquitin ligase will be discovered and developed as a novel class of anticancer drugs. E3-based cancer biomarkers will be also developed and used in clinics as diagnostic tools or prognostic indices for the benefit of cancer patients.

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