Proteins involved in Cell Communication as Target In Treatment

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Introduction

- Cancer is characterized by uncontrolled proliferation resulting from aberrant activity of various cell cycle proteins.

- Cell cycle regulators proteins are considered attractive targets in cancer therapy.

- After decades of research on the physiological functions of cell cycle proteins and their relevance for cancer, this knowledge recently translated into approved cancer therapeutic targeting of a direct regulator of the cell cycle.

Cell cycle progression and major regulatory proteins

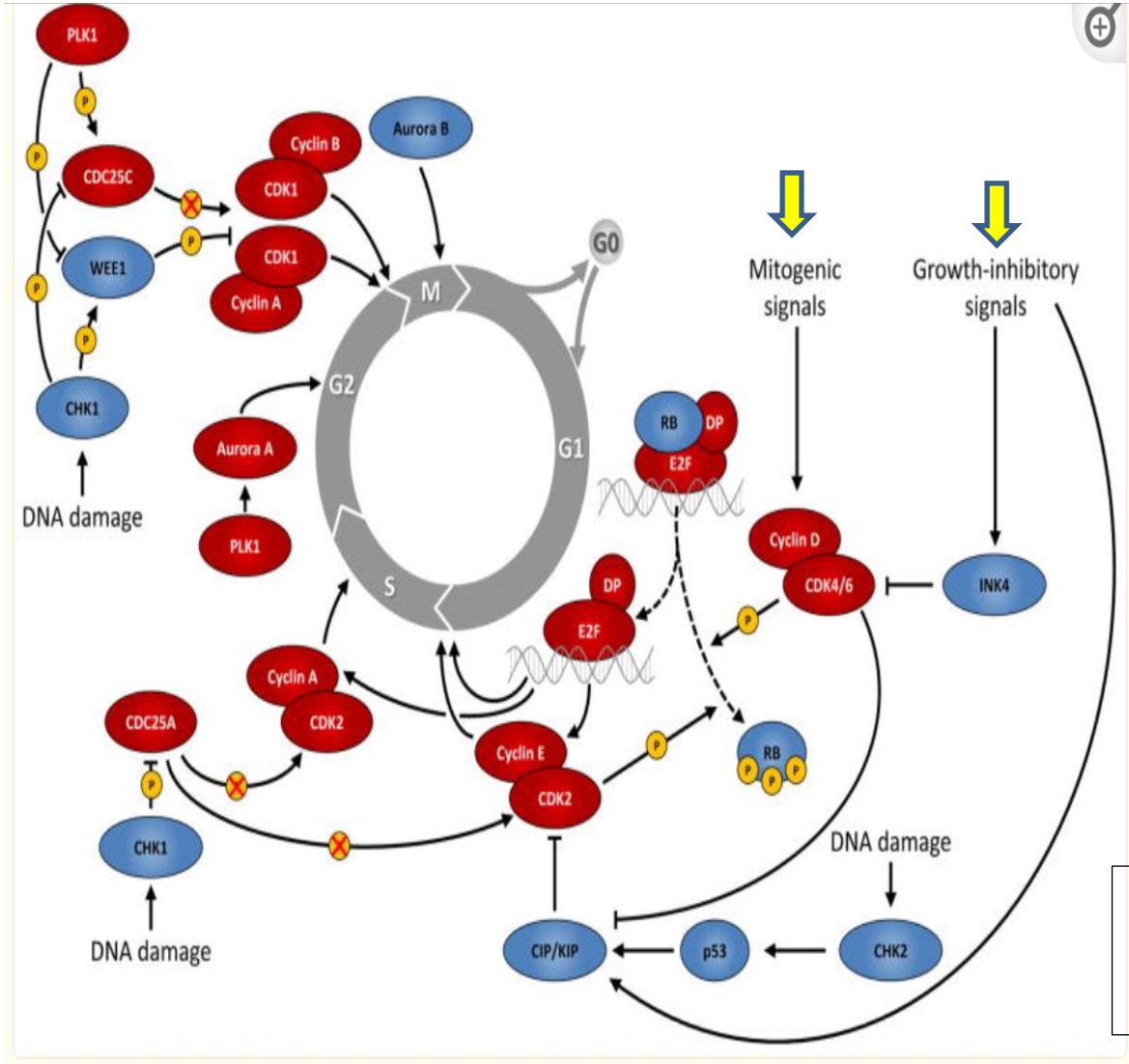
- The mammalian cell cycle is a highly organized and regulated process that ensures duplication of genetic material and cell division.

- This regulation involves growth-regulatory signals as well as signals by proteins monitoring the genetic integrity to ascertain the absence of any genetic damage.

- Proliferation depends on progression through four distinct phases of the cell cycle (G0/G1, S, G2 and M), which is regulated by several cyclin-dependent kinases (CDKs) that act in complex with their cyclin partners.

- The activity of CDKs involved in cell cycle regulation is tightly controlled; it is induced by mitogenic signals and can be inhibited by activation of cell cycle checkpoints in response to DNA damage .

Cell cycle progression and major regulatory proteins

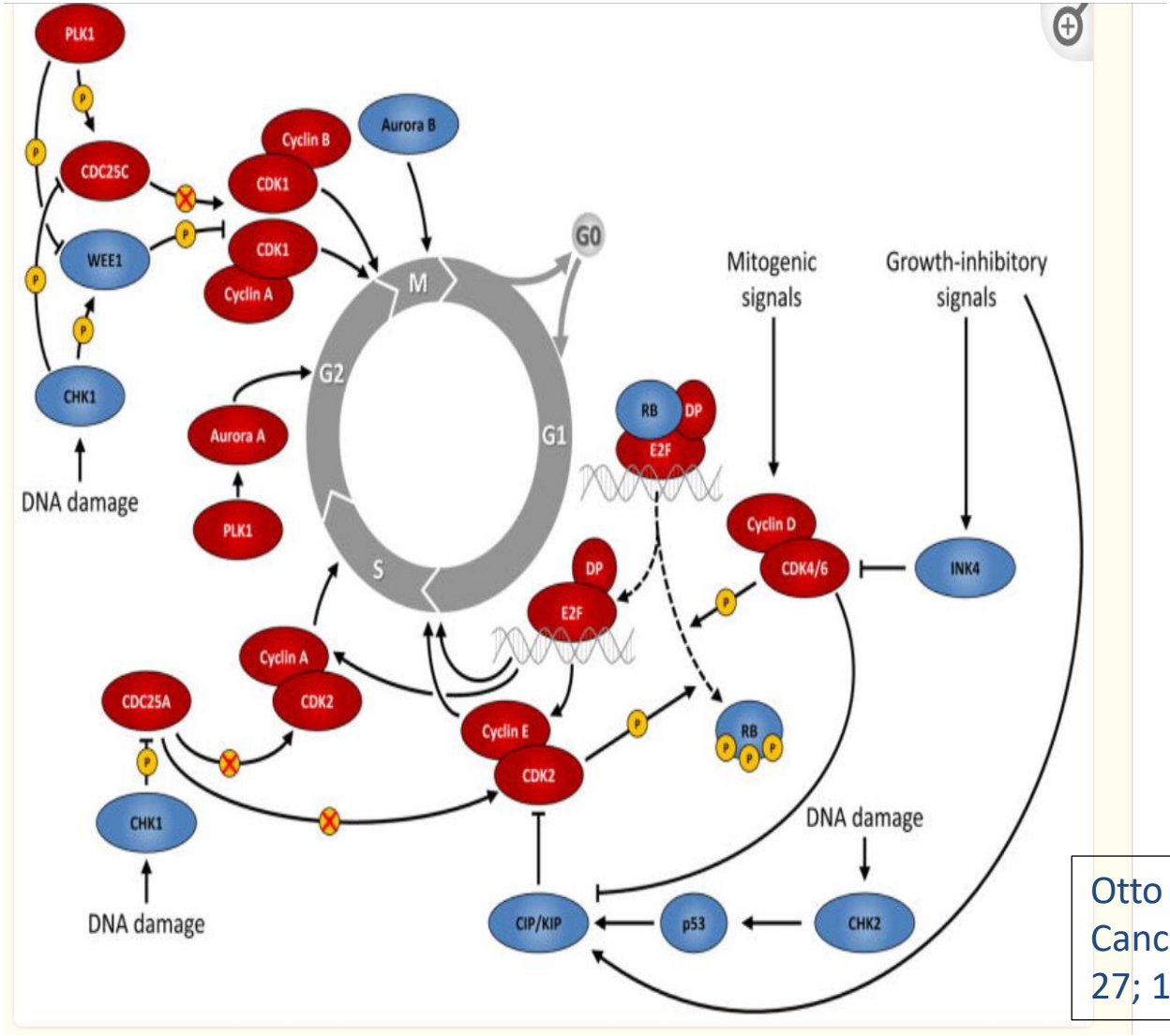


Otto T et al. Nat Rev Cancer. 2017 January 27; 17(2): 93–115

Mitogenic signals activate complexes of cyclins and cyclin-dependent kinases (CDKs) that promote progression from the G1 phase into S phase mainly by phosphorylating the retinoblastoma protein (RB) and subsequent activation of transcription by the E2F family of transcription factors.

Growth-inhibitory signals antagonize G1-S progression by upregulating CDK inhibitors of the INK4 and CIP/KIP families.

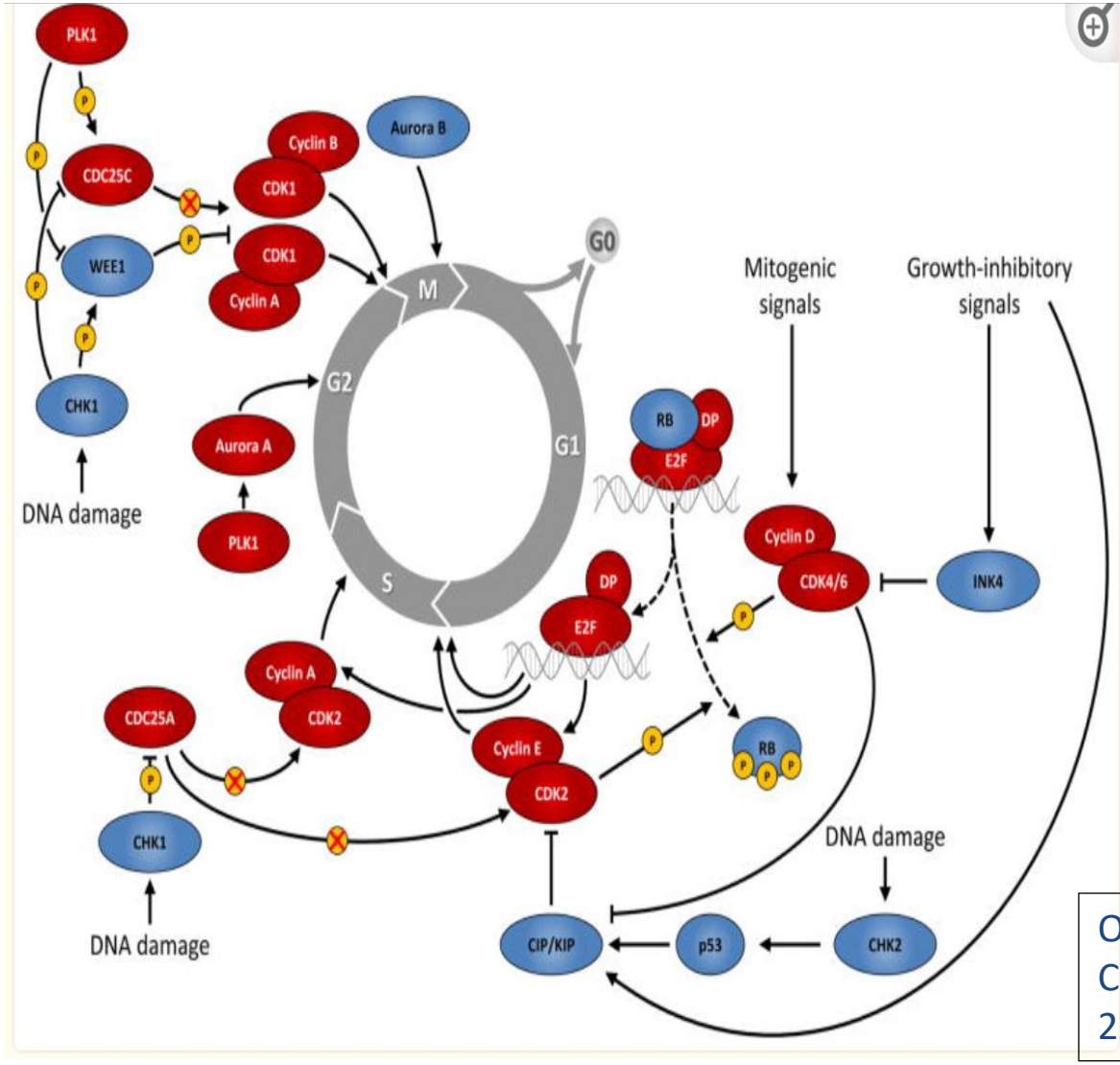
Cell cycle progression and major regulatory proteins



Otto T et al. Nat Rev Cancer. 2017 January 27; 17(2): 93–115

Progression through S phase and from G2 phase into mitosis (M phase) is also controlled by cyclin-CDK complexes, together with a variety of other proteins, such as Polo-like kinase 1 (PLK1) and Aurora kinases (Aurora A/B).
Cells can also exit the cell cycle and enter a reversible or permanent cell cycle arrest (G0 phase).

Cell cycle progression and major regulatory proteins



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In addition, DNA damage is sensed by several specialized proteins and triggers cell cycle arrest via checkpoint kinase 2 (CHK2) and p53 in G1 phase or via checkpoint kinase 1 (CHK1) in S or G2 phase.
Red and blue ovals denote positive and negative regulators of cell cycle progression, respectively.

Cell cycle proteins and their role in Physiology and Cancer

The biology of the CDK4/CDK6-RB pathway

- In most adult tissues, cells are residing in a cell cycle arrested state termed G0 phase, which can be either transient (quiescence) or permanent (upon terminal differentiation or senescence).

- Quiescent cells can be triggered to re-enter the cell cycle through stimulation with mitogenic factors.

- Most of these factors activate cascades of intracellular signalling networks and impinge on CDK4 and CDK6 to drive cell cycle progression from G0/G1 into S phase, in which DNA replication occurs.

- The activity of CDK4 and CDK6 is controlled by several mechanisms: positively by association with D-type cyclins (D1, D2 and D3) and negatively by binding to CDK inhibitors of the INK4 family ($p16^{INK4A}$, $p15^{INKB}$, $p18^{INK4C}$ and $p19^{INK4D}$)

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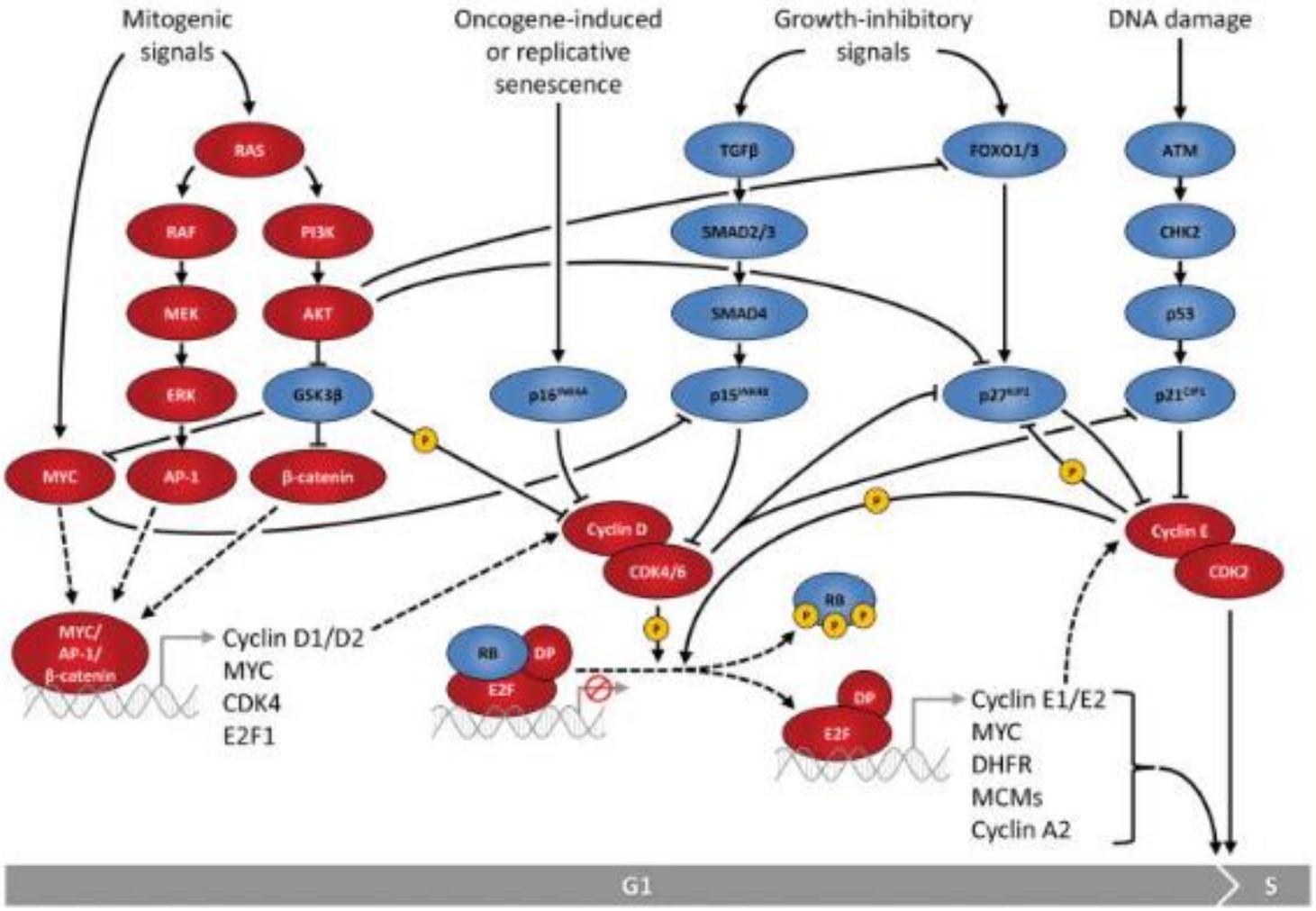
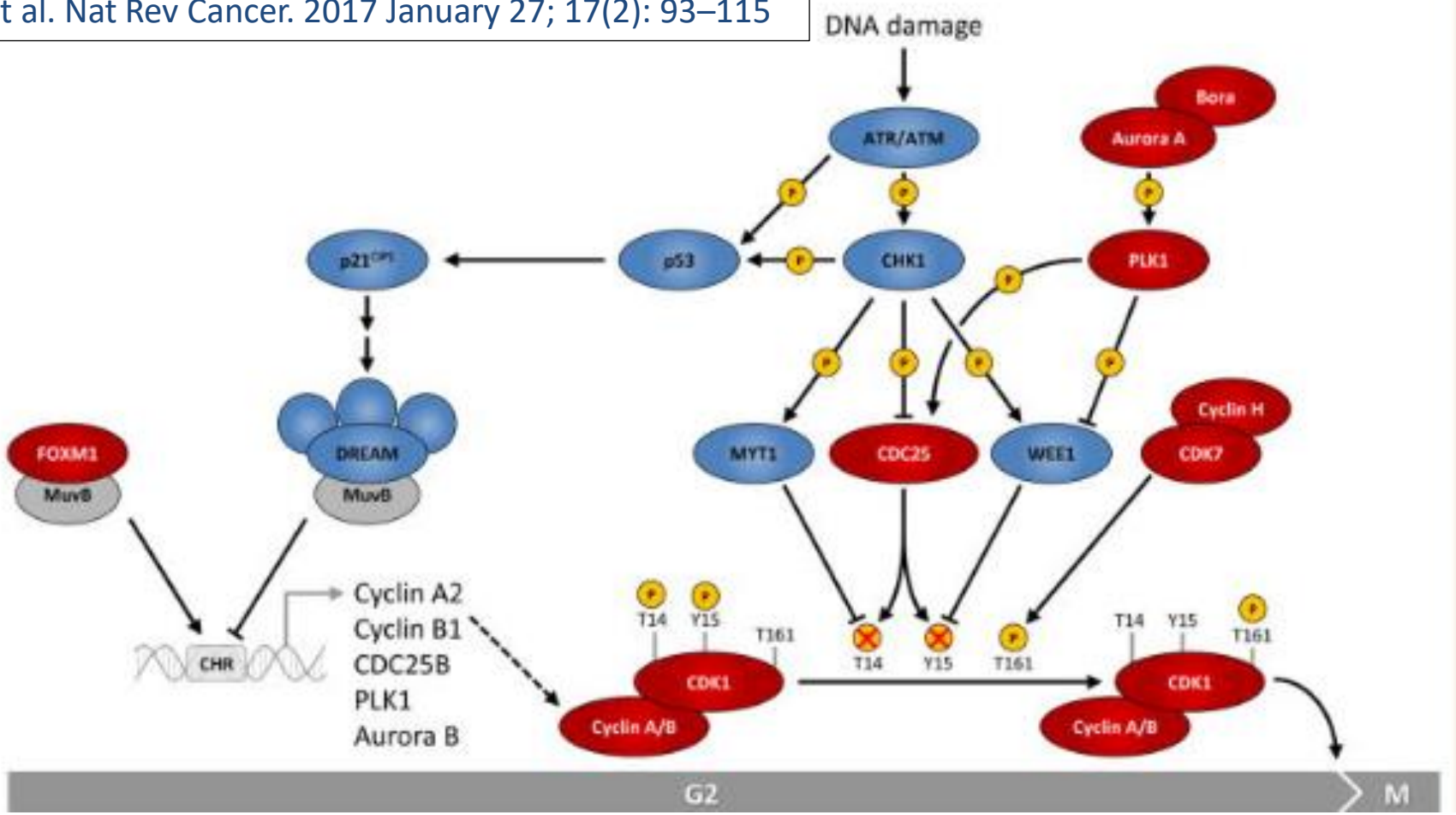


- Entry into the cell cycle is typically induced in response to mitogenic signals that activate signalling pathways such as the RAS pathway.
- These pathways eventually impinge on transcriptions factors such as MYC, AP-1 or β-catenin and lead to induction of a number of cell cycle proteins including D-type cyclins.
- Formation of active complexes of D-type cyclins and cyclin-dependent kinases (CDKs) 4 and 6 drives phosphorylation of the RB (retinoblastoma) protein and is antagonized by the INK4 family (p16INK4A and p15INK4B) in response to senescence-inducing or growth-inhibitory signals, such as the transforming growth factor β (TGFβ).

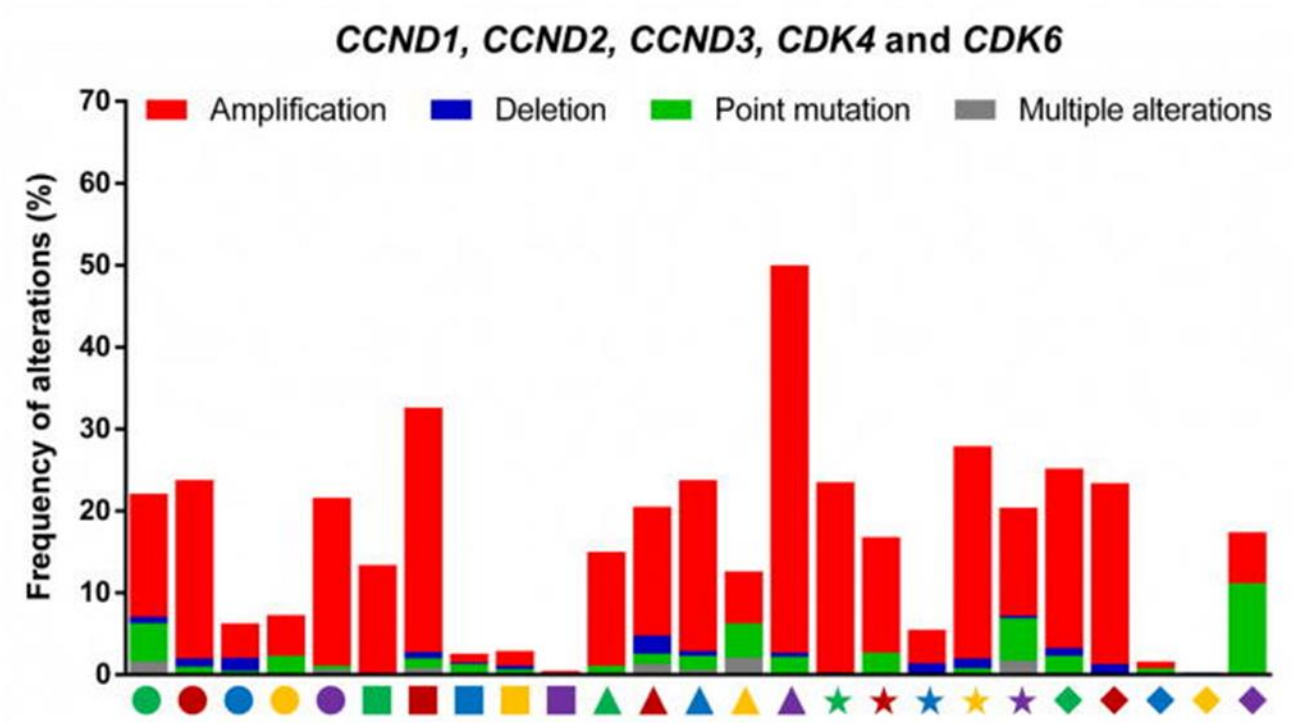
Otto T et al. Nat Rev Cancer. 2017 January 27; 17(2): 93–115



- During G2 phase, the MuvB complex associates with the transcription factor FOXM1 and binds promoters containing cell cycle genes homology region (CHR) elements, thereby inducing transcription of genes required for entry into and progression through mitosis (M phase), including B-type cyclins.
- Activation of cyclin B-CDK1 kinase requires phosphorylation of CDK1 at Thr-161 by the cyclin H-CDK7 complex (CAK, CDK-activating kinase) as well as dephosphorylation of Thr-14 and Tyr-15 on CDK1 by cell division cycle 25 (CDC25) family phosphatases, the latter process being antagonized by protein kinases MYT1 and WEE1.

The role of the CDK4/CDK6-RB pathway in cancer

- Components of the CDK4/6-RB Pathway are commonly mutated in human cancers



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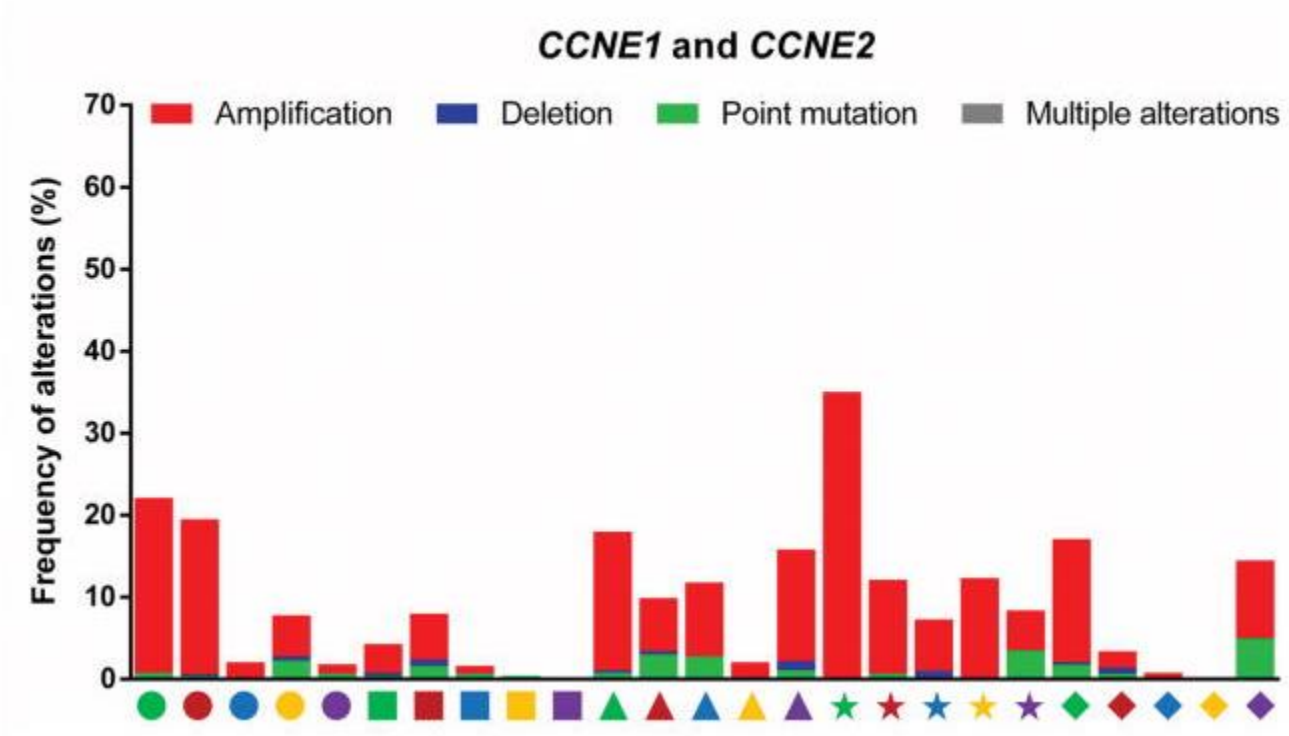

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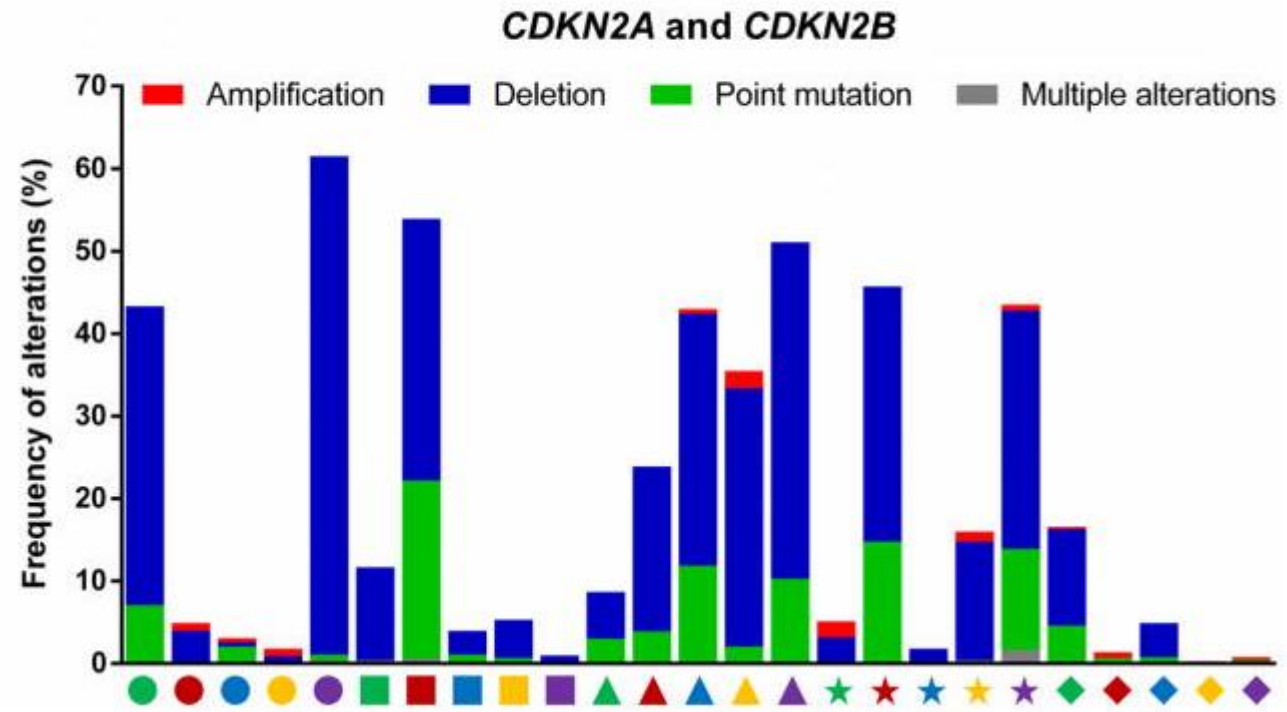

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The role of the CDK4/CDK6-RB pathway in cancer

- The cyclin D1 gene (CCND1) represents the second most frequently amplified locus among all human cancer types.
- CDK4 is amplified in 50% of glioblastomas and constitutively activated by a point mutation (R24C, which renders CDK4 insensitive to inhibition by INK4 family members) in melanomas.
- Similarly, CDK6 is activated by genomic translocations in splenic marginal zone lymphomas
- Furthermore, the CDKN2A gene (which encodes the tumour suppressors p16INK4A and p14ARF) represents the most frequently deleted locus in human cancers and its expression is also commonly silenced by promoter methylation.
- Finally, deletion of the retinoblastoma gene (RB1) occurs frequently in many tumour types and allows proliferation independently of cyclin D-CDK4/6 activity.

Beroukhim R et al.. Nature. 2010; 463:899–905
Hydbring P et al. Nat Rev Mol Cell Biol. 2016; 17:280–92

Targeting of other cell cycle proteins
Inhibitors of CHK1 and WEE1

- During the last decade, a number of CHK1 and WEE1 inhibitors have been developed and tested in preclinical and clinical studies.
- Currently, three of them seem promising: the CHK1 inhibitors MK-8776 and LY2606368 and the WEE1 inhibitor AZD1775
- MK-8776 exhibits high potency and selectivity for CHK1182.
- Treatment of cancer cells with MK-8776 caused accumulation of DNA double-strand breaks leading to apoptotic cell death in vitro.
- Furthermore, it synergized with gemcitabine, hydroxyurea and cytarabine in causing apoptosis of AML and breast cancer cells in vitro, as well as with gemcitabine in ovarian and pancreatic cancer xenografts

Guzi TJ, et al. Mol Cancer Ther. 2011; 10:591–602.
Schenk EL, et al. Clin Cancer Res. 2012; 18:5364–73.

Inhibitors of Polo-like kinases

- Development of Polo-like kinase inhibitors has mainly focussed on PLK1.
- Currently, two promising PLK1 inhibitors, rigosertib and volasertib, are under clinical investigation.
- Rigosertib is a multi-kinase inhibitor with highest affinity for PLK1205.
- Rigosertib caused tumour regression in a xenograft model of head and neck squamous cell carcinoma and (in combination with gamma-radiation) in xenografts of cervical cancer.

Anderson RT, et al. Mol Cancer Ther. 2013; 12:1994–2005.
Agoni L, et al. Int J Radiat Oncol Biol Phys. 2014; 88:1180–7.

AURORA –A Kinase (AKA)

- Aurora-A regulates cell cycle progression by regulating the spindle and mitotic checkpoints.
- Its main functions are mitotic regulation, promotion of mitotic entry, and cell growth arrest .
- Overexpression of Aurora-A is linked to breast, ovary, and colon tumors
- Moreover, overexpression of Aurora-A has been associated with radio- and chemoresistance in laryngeal cancer cells,cervical cancer and breast cancer.
- Aurora-A has been suggested to induce chemoresistance in several cancers by reducing apoptosis via activation of the NF-κB/miR-21/PTEN (phosphatase and tensin homolog) signaling pathway and Akt through inhibition of the p53/PTEN cascade.

Sehdev V, et al. Mol Cancer Ther. 2012; 11:763–74
Melichar B, et al. Lancet Oncol. 2015; 16:395–405

AURORA –A Kinase Inhibitor

- Aurora-A kinase inhibitors currently used in preclinical and clinical studies include MLN8054, PF-03814735, AS703569, MK-0457, MK-5108, MSC1992371A and MLN8237.

- Small molecule inhibitors MLN8237 was effective in treating acute myelogenous leukemia and chronic myelogenous leukemia in Phase II trials when used in combination with cytarabine and nilotinib

- Another Aurora-A kinase inhibitor, MK-5108 (Phase I), inhibits cell growth and induces G2/M arrest in chemoresistant epithelial ovarian cancer stem cells by affecting the NF-κB pathway

- A number of inhibitors targeting the major family members Aurora A or Aurora B, such as alisertib, ENMD-2076, danusertib and AMG-900, have been developed and are under clinical investigation.

Melichar B, et al. Lancet Oncol. 2015; 16:395–405
Barr PM, et al. J Clin Oncol. 2015; 33:2399–404

Rationale for targeting specific cell cycle proteins

- Cell cycle proteins are frequently overactive in cancer cells leading to uncontrolled proliferation.

- Genetic ablation of individual cyclins or CDKs, or inhibition of cyclin-CDK kinase activity in tumor-bearing mice selectively blocked tumor initiation and progression of specific cancer types driven by particular oncogenic insults, without having major effects on normal tissues.

- This suggests that tumor cells are dependent on (or “addicted” to) specific CDKs, depending on genetic lesions they carry, and hence CDK inhibition may selectively target cancer cells while sparing normal tissues.

- In some instances, inhibition of CDK activity in mouse cancer models not only led to cell cycle arrest but also provoked tumor cell senescence or apoptosis.

- This indicates that tumor carrying particular genetic lesions critically depend on specific cell cycle proteins to inhibit **tumor - suppressive programs such as senescence and apoptosis**, thereby selectively sensitizing cancer cells to inhibition of these proteins.

Targeting CDKs in cancer therapy

Development of pan-CDK inhibitors

- Most of the early compounds exhibited little specificity towards individual CDKs and are therefore commonly referred to as pan-CDK inhibitors.
- The first generation of these inhibitors include flavopiridol, (R)-roscovitine and olomoucine.
- Flavopiridol is a semisynthetic flavone targeting many CDKs and represents the most extensively studied CDK inhibitor with over 60 clinical trials initiated since 1997.
- It causes cell cycle arrest in G1 and G2 phases. Administration of flavopiridol induced apoptosis in several mouse tissues leading to organ atrophy, an effect attributed to inhibition of cyclin T1-CDK9 (P-TEFb) kinase.
- Although flavopiridol exhibited significant anti-tumour activity in preclinical studies, clinical phase II studies reported insufficient efficacy for solid cancers. However, some evidence for clinical activity was observed in haematological malignancies

Lin TS, et al. Clin Oncol. 2009; 27:6012–8
Lanasa MC, et al. Leuk Res. 2015; 39:495–500

Development and clinical success of CDK4/CDK6-selective inhibitors

- Following promising results from genetic and preclinical studies, the first group of CDK-selective compounds that entered the clinics were CDK4/6 inhibitors Palbociclib, Ribociclib and Abemaciclib

- Palbociclib was originally developed by David Fry and Peter Toogood in 2001, although it took many years until its potential therapeutic value became appreciated, and phase II clinical trials eventually started in 2009130. Palbociclib potently inhibits both CDK4 and CDK6 kinase activity whereas other kinases are barely affected.

Sherr CJ, et al. Cancer Discov. 2015
Garber K. Science. 2014; 345:865–7

- As expected, Palbociclib prevents RB phosphorylation by CDK4/6 and causes cell cycle arrest in G1 phase.

- Analyses of human cancer xenografts demonstrated a strong anti-tumour activity against glioblastomas, colorectal cancers, rhabdomyosarcomas multiple myelomas, AML, ALL and dermatofibrosarcomas.

- In search for cancer types particularly sensitive to Palbociclib, Finn and colleagues demonstrated that luminal-type breast cancer cells expressing oestrogen receptor ER+), including luminal-type cells with amplification of the HER2 receptor were significantly more sensitive to Palbociclib than ER-negative (ER-) breast cancer cells with basal-like histology.

Fry DW, et al. Mol Cancer Ther. 2004; 3:1427–38
Dean JL et al. Oncogene. 2010; 29:4018–32

CDK4/6 Inhibitor

PALOMA 3 Trial

- Palbociclib and Fulvestrant (an ER antagonist) Vs placebo and fulvestrant for women with ER+ HER2- metastatic breast cancer that have relapsed or progressed during prior hormone therapy, Including a substantial portion of patients (33%) with prior chemotherapy for metastatic disease.

- The interim analysis of this study demonstrated a significantly improved median PFS (9.5 months Vs 4.6 months, respectively)

Synthetic lethal targets

- Experimental approaches from yeast genetics led to screens that identify proteins whose activities are essential for cancers harboring specific oncogenic mutations.

- Synthetic lethality allows targeting of cancers that harbor mutations in undruggable proteins with potential to improve therapeutic index.

- The success of poly (ADP-ribose) polymerase (PARP) inhibition in the setting of BRCA1 and BRCA2 deficiency, for instance, provided an important proof of principle
- Inhibition of PARP capitalized on defects in DNA repair induced by loss of BRCA1/2 and other homologous recombination DNA repair mediators, leading to mitotic catastrophe.

- One mechanism of resistance to PARP inhibition is the reversion of BRCA1 to produce a wild-type protein, confirming a direct relationship between BRCA1 mutation and PARP inhibitor sensitivity

Lord C J et al. Science. 2017 Mar 17;355(6330):1152-1158

Emerging Cancer Targets

Protein-protein interactions

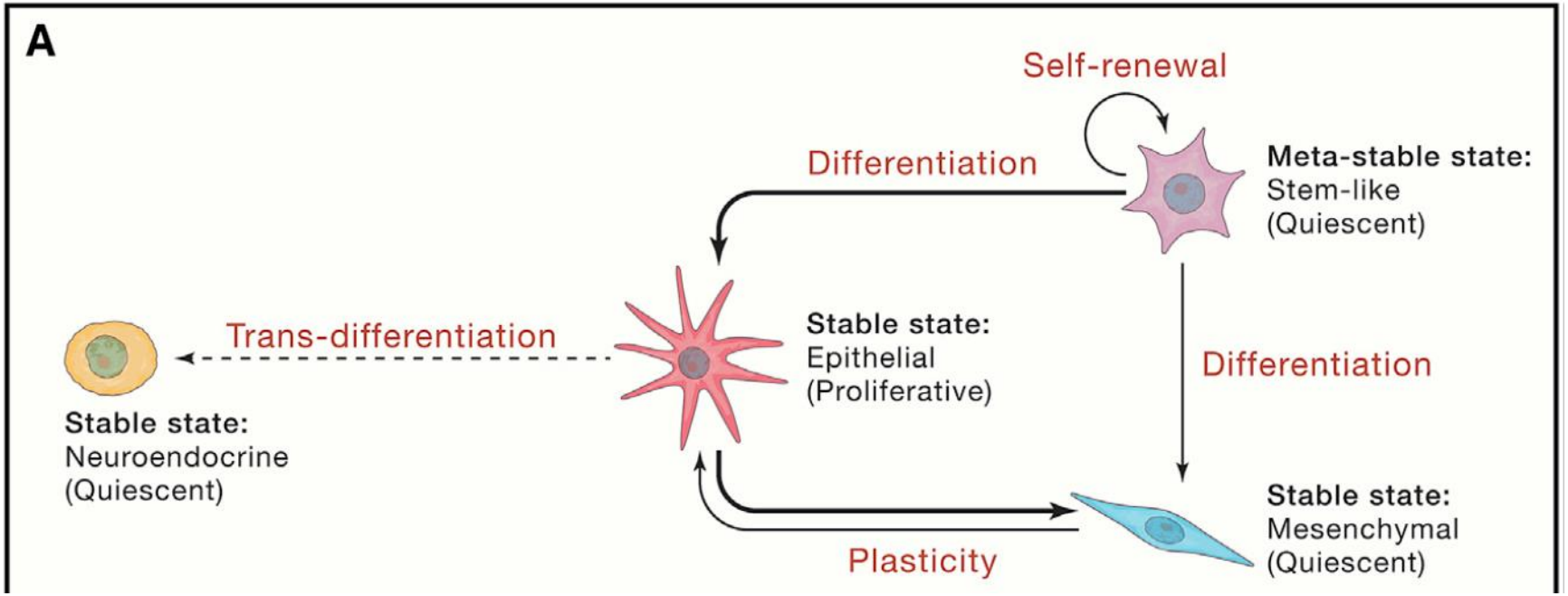
- Oncogenic missense mutations can alter protein-protein interactions and drive cancer progression.

- Thus, identifying and characterizing critical nodes and hubs in cancer-related protein-protein interaction networks, which control the output of oncogenic programs, may reveal unique opportunities for therapeutic intervention.

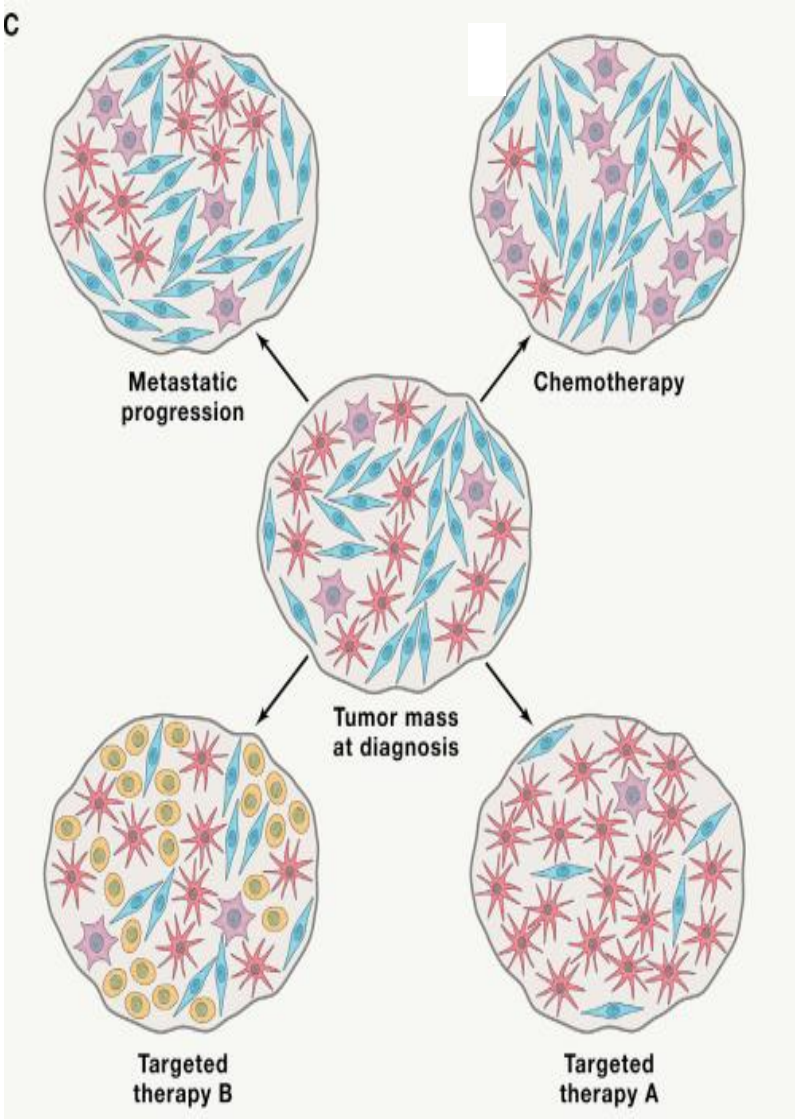
- In particular, the systematic interrogation of new protein-protein interactions driven by mutant oncoproteins may reveal new types of cancer targets that are cancer specific.

Oncogenic targets and cell states

- Schematic representation of isogenic tumor cells presenting with distinct transcriptional states and epigenetic profiles.



Schematic representation of the tumor composition changes following drug treatment or spontaneous progression



Tumor Micro Environment (TME)

- The TME is a rich milieu in which diverse non-neoplastic cell types and extracellular matrix proteins interact to regulate the biology of cancer cells.

- Deeper mechanistic insights into these dynamic molecular exchanges have enabled therapeutic strategies directly targeting aspects of the TME that are essential for tumor function.

- Proof-of-principle for TME-directed cancer therapy was achieved by anti-angiogenic treatments targeting vascular endothelial growth factor (VEGF), inhibiting the tumor's recruitment of new blood vessels and exploiting the relative lack of neovascularization in healthy organs

- The intricate cellular complexity of the TME spans immune cells, fibroblasts, extracellular matrix (ECM) and even neuronal elements creating a multitude of potential targets.

Monje, 2020, Venkatesh et al., 2019

Tumor Micro Environment (TME)

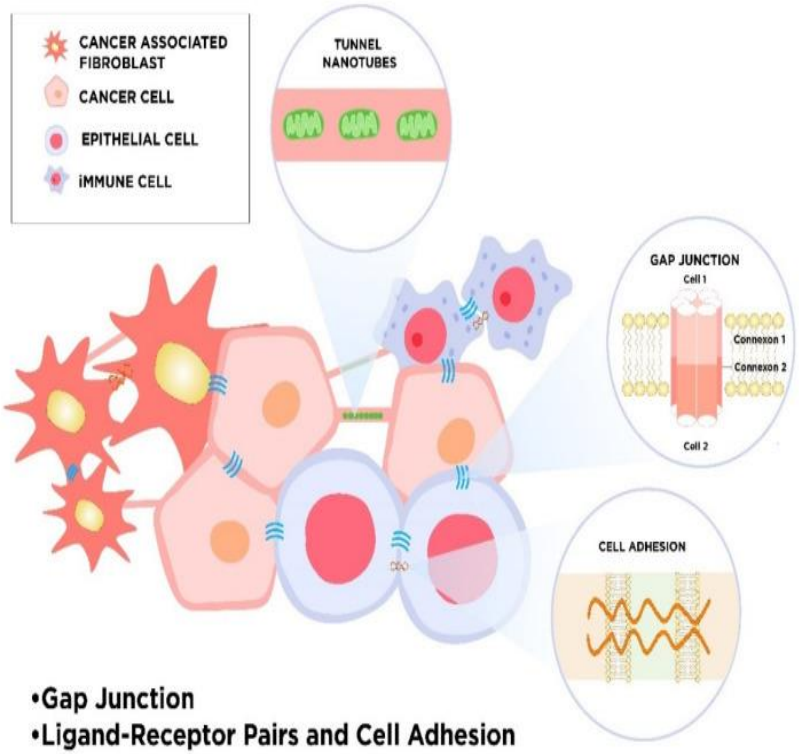
The TME is a rich milieu in which diverse non-neoplastic cell types and extracellular matrix proteins interact to regulate the biology of cancer cells.

Cross-talk within the cancer microenvironment, cell-to-cell contact

1. Adhesion molecules
2. Electrical coupling
3. Passage through gap junctions
4. Indirect through classical paracrine signaling by cytokines, growth factors, and extracellular vesicles.

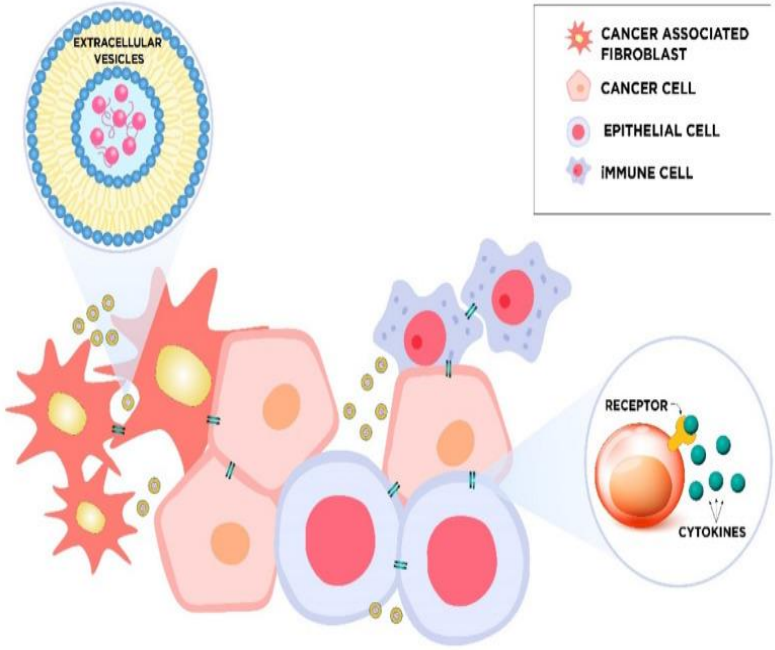
Therapeutic approaches for modulation of cell-cell communication may be a promising strategy to combat tumors.

DIRECT COMMUNICATION



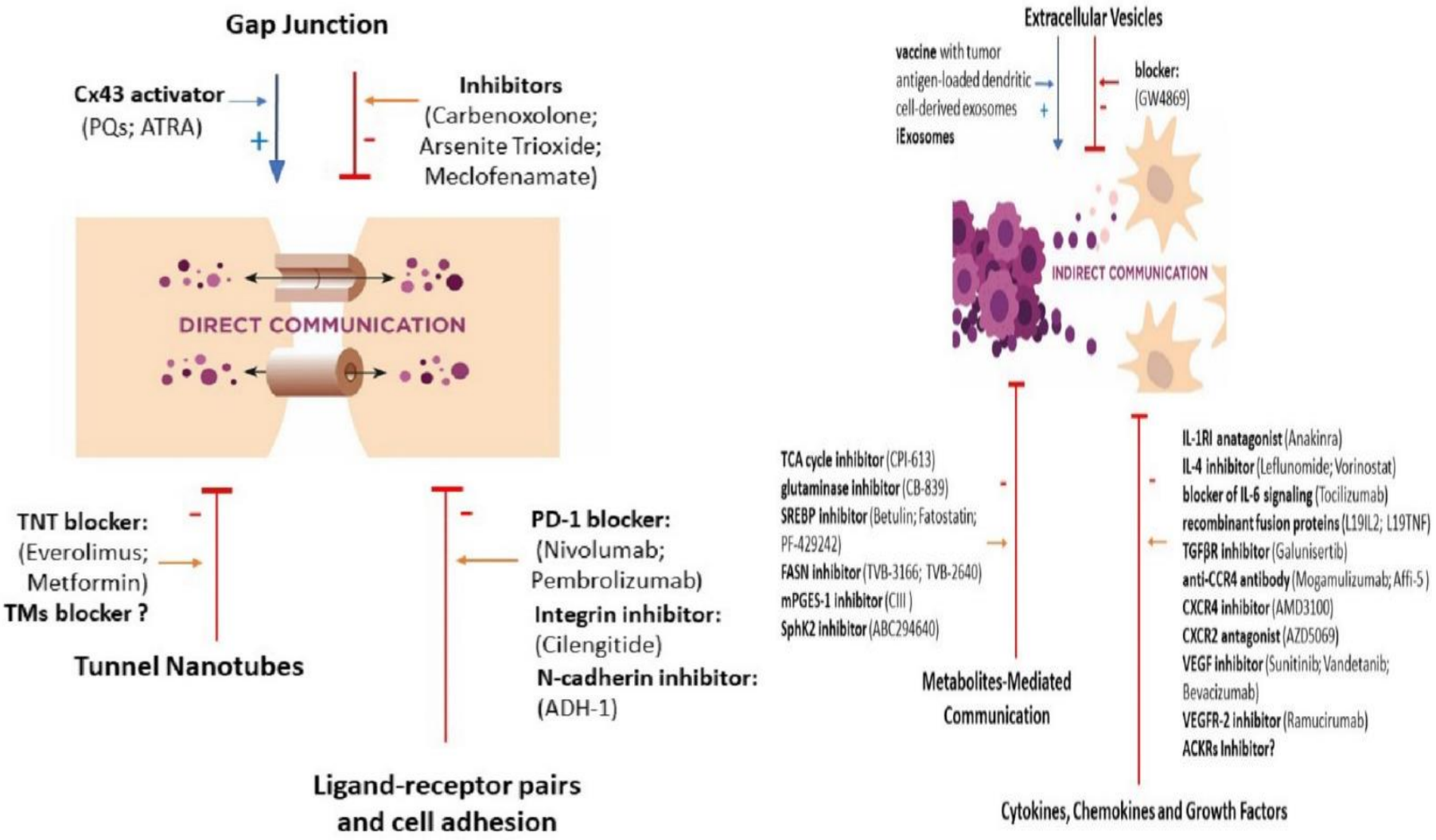
- Gap Junction
- Ligand-Receptor Pairs and Cell Adhesion
- Tunnel Nanotubes

INDIRECT COMMUNICATION



- Signaling by Extracellular Vesicles
- Signaling by Cytokines, Chemokines and Growth Factors
- Metabolites-Mediated Communication

Communication in the Cancer Microenvironment as a Target for Therapeutic Interventions



Cancers (Basel). 2020 May; 12(5): 1232

Chromatin-regulating proteins as targets for cancer therapy

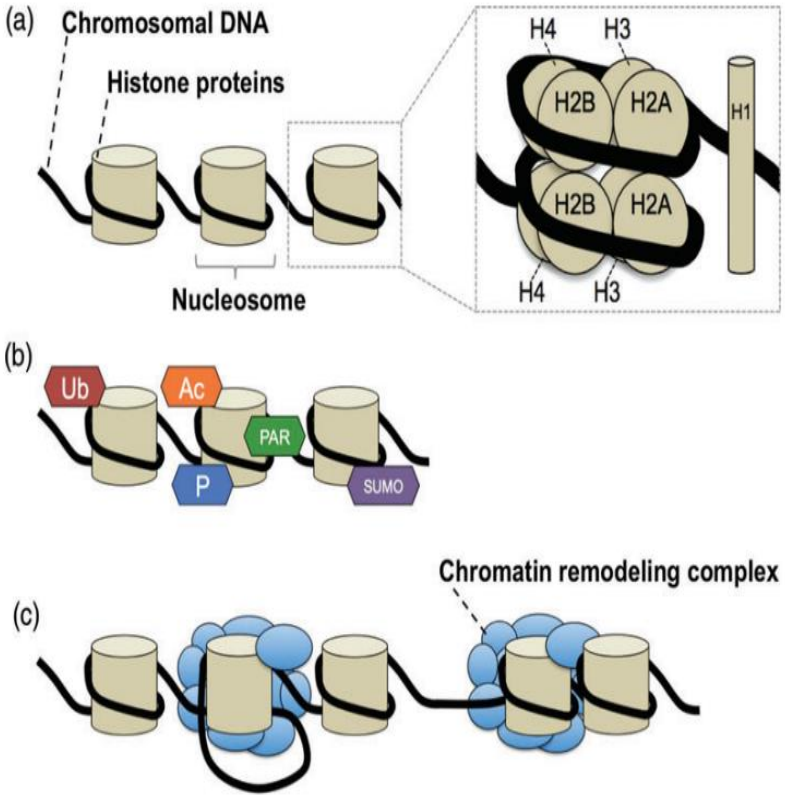


Fig. 1. Chromatin structure and its alteration by two distinct mechanisms: histone modification and chromatin remodeling. (**a**) Structure of chromatin and nucleosome. (**b**) Examples of histone modification. Ac = acetylation, PAR = poly-ADP-ribosylation, P = phosphorylation, SUMO = SUMOylation, Ub = ubiquitination. (**c**) Chromatin remodeling: DNA-loop formation (left) and nucleosome sliding (right).

Oike, T et al. J Radiat Res, 2014, 55, 613–628

BRCA 1/2

- The tumor suppressor genes BRCA1/2 - involved in several cellular mechanisms such as cell cycle checkpoint control, chromosome remodeling, transcriptional regulation, DNA repair, and apoptosis [79,81]

- BRCA1/2 are essential for both S and G2/M checkpoints in response to DNA damage caused by either radio or chemotherapy, and play important roles in multiple DNA repair pathways such as homologous recombination (HR) and transcription-coupled nucleotide excision repair.

- Therefore, BRCA1/2-null cancers are more sensitive to platinum-based DNA damaging agents and to PARP inhibitor

- Mutations in p53 upregulate BRCA1 and induce resistance to Cisplatin in breast cancer.

Farmer, H et al. Nature 2005, 434, 917–921

BRCA 1/2 (contd.)

- BRCA1 can activate the transcriptional target TDP2 that pairs with ETS2 and mediates etoposide resistance in mutp53-bearing cells.

- Inhibition of the homologous recombination (HR) pathway proteins RAD52/51 with small molecule D-I03 can specifically inhibit the biochemical activities of RAD52 and suppress growth of BRCA1 and BRCA2 null cells .

- PARP inhibitors are also capable of sensitizing tumor cells with impaired HR activity by genomic instability and cell death.

- Since BRCA1 and BRCA2 mutated cells lack HR pathways, such inhibitors improve the effectiveness of chemotherapy in breast and ovarian cancer treatment.

Huang, F. et al. *Nucleic Acids Res.* **2016**, *44*, 4189–4199

WNT Signalling

- Upregulation of WNT5A is associated with breast cancer, prostate cancer, melanoma and pancreatic cancer indicating its oncogenic role in these cancers.

- WNT5A is thought to induce chemoresistance in pancreatic cancer through enhanced PI3K/Akt signalling that affects the G1/S phase transition

- WNT5A was highly expressed in BRAF inhibitor (BRAFi)-resistant melanoma tumors

Anastas, J.N et al. J. Clin. Investig. 2014, 124, 2877–2890

WNT Signalling (Cont.)

- WNT5A knockdown showed an increase of cells in G0/G1 phase and a decreased cell number in S phase, which enhanced the chemosensitivity of pancreatic cancer cells to gemcitabine

- WNT5A contributed to drug-resistance by enhancing anti-apoptosis ability in pancreatic cancer cells

- WNT5A mediated gemcitabine chemoresistance was via the regulation of cell cycle, a target for chemotherapeutic response in pancreatic cancer.

Anastas, J.N et al. J. Clin. Investig. 2014, 124, 2877–2890

Check Point Inhibitor

- **PD-1 inhibitors**

Examples of drugs that target PD-1 include:

- Pembrolizumab
- Nivolumab
- Cemiplimab

- **PD-L1 inhibitors**

Examples of drugs that target PD-L1 include:

- Atezolizumab
- Avelumab
- Durvalumab

- **CTLA- 4 inhibitors**

Ipilimumab & Tremelimumab

- **LAG-3 inhibitors**

Relatlimab

Apoptosis as Target

There are three main known mechanisms by which cancer cells acquire apoptosis resistance:

(1) a disruption in the balance between pro- and anti-apoptotic proteins

(2) an impairment of signalling through death receptors

(3) a reduction in the function of caspases

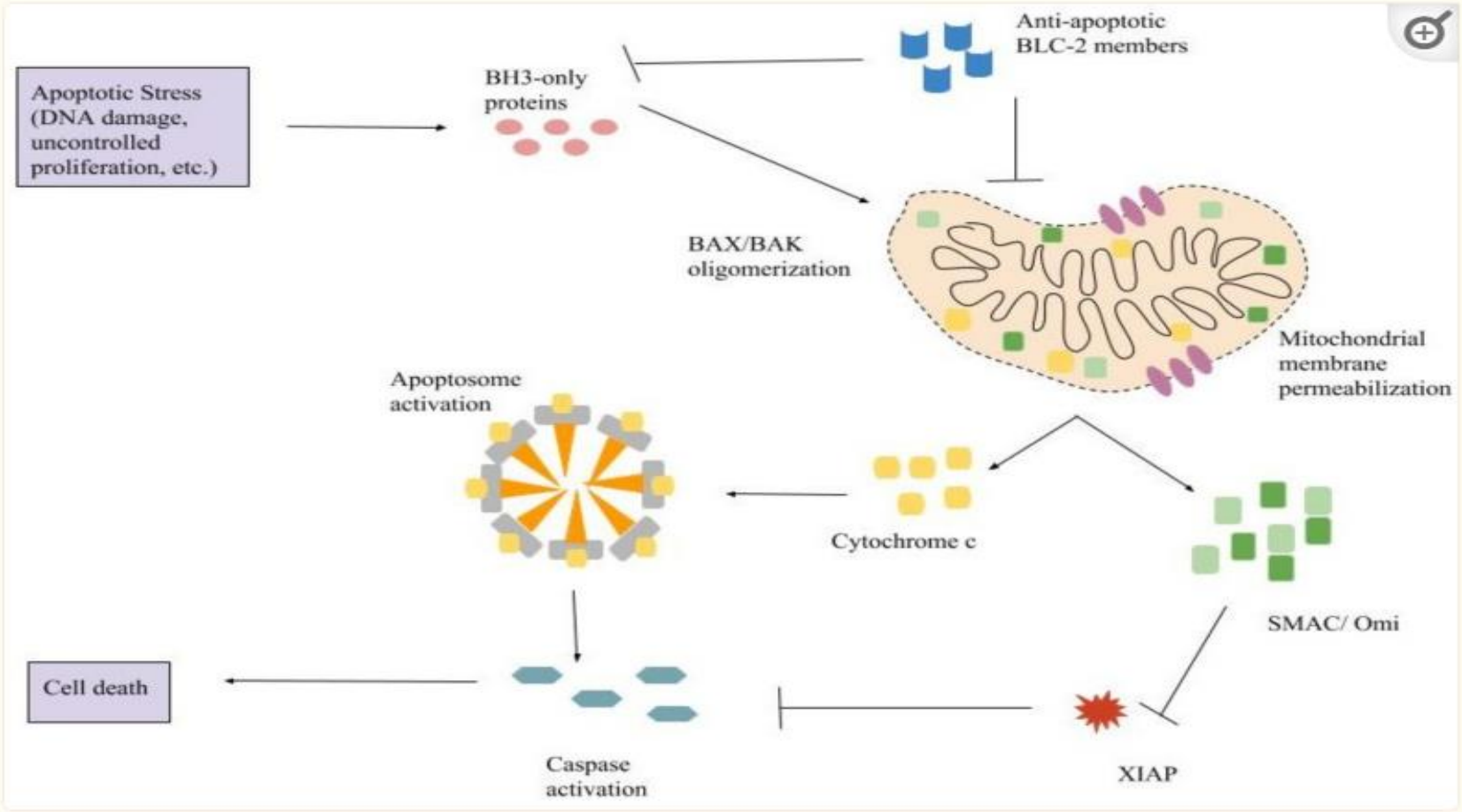


Figure 1

The pathway of intrinsic apoptosis. BH3-only proteins are upregulated in response to apoptotic stress. They activate BAX (BCL-2-associated X protein) and BAK (BCL-2 homologous antagonist killer) which oligomerize and results in mitochondrial membrane permeabilization. Cytochrome c, SMAC (second mitochondria-derived activator of caspase), and Omi are released and the apoptosome is formed from procaspase-9, dATP, cytochrome c, and APAF-1. Caspases are then activated and begin to cleave cellular proteins resulting in apoptosis. Arrows represent activation and T bars represent inhibition.

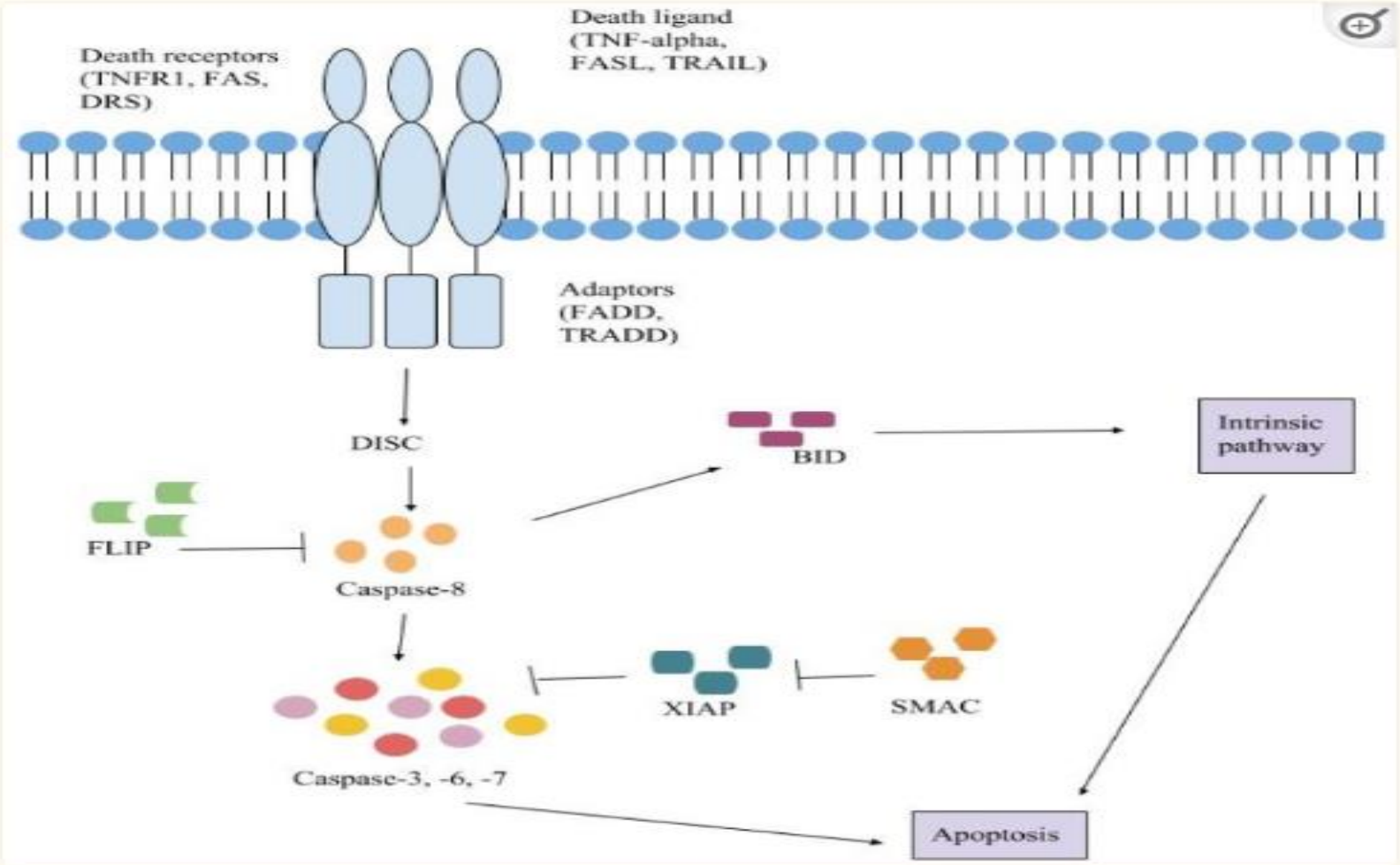


Figure 2

The extrinsic pathway begins with a death ligand docking on a death receptor. An adaptor protein binds to the receptor. DISC (death-inducing signaling complex) is formed from the adaptor protein and procaspases-8 and -10. Caspase-8 becomes activated which activates caspases-3, -6 and -7 and BID (BH3 interacting-domain death agonist). BID goes on to activate BAX and BAK which activates the intrinsic pathway. Caspases-3, -6 and -7 are the executioner caspases that result in cell death. Arrows represent activation and T bars represent inhibition.

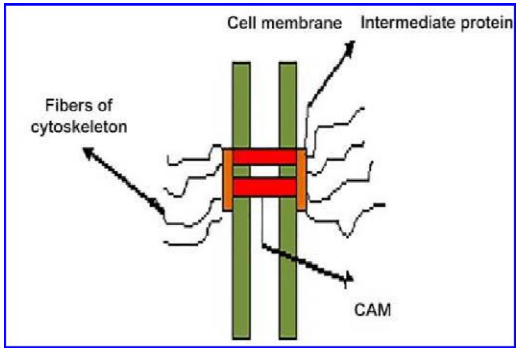
Cell Adhesion Molecule (CAM) as Target

- Cell adhesion molecules (CAMs) are a subset of cell surface proteins[1] that are involved in the binding of cells with other cells or with the extracellular matrix (ECM), in a process called cell adhesion.[2]

- CAM function in cancer metastasis, inflammation, and thrombosis makes it a viable therapeutic target that is currently being considered.

- For example, they block the metastatic cancer cells' ability to extravasate and home to secondary sites.

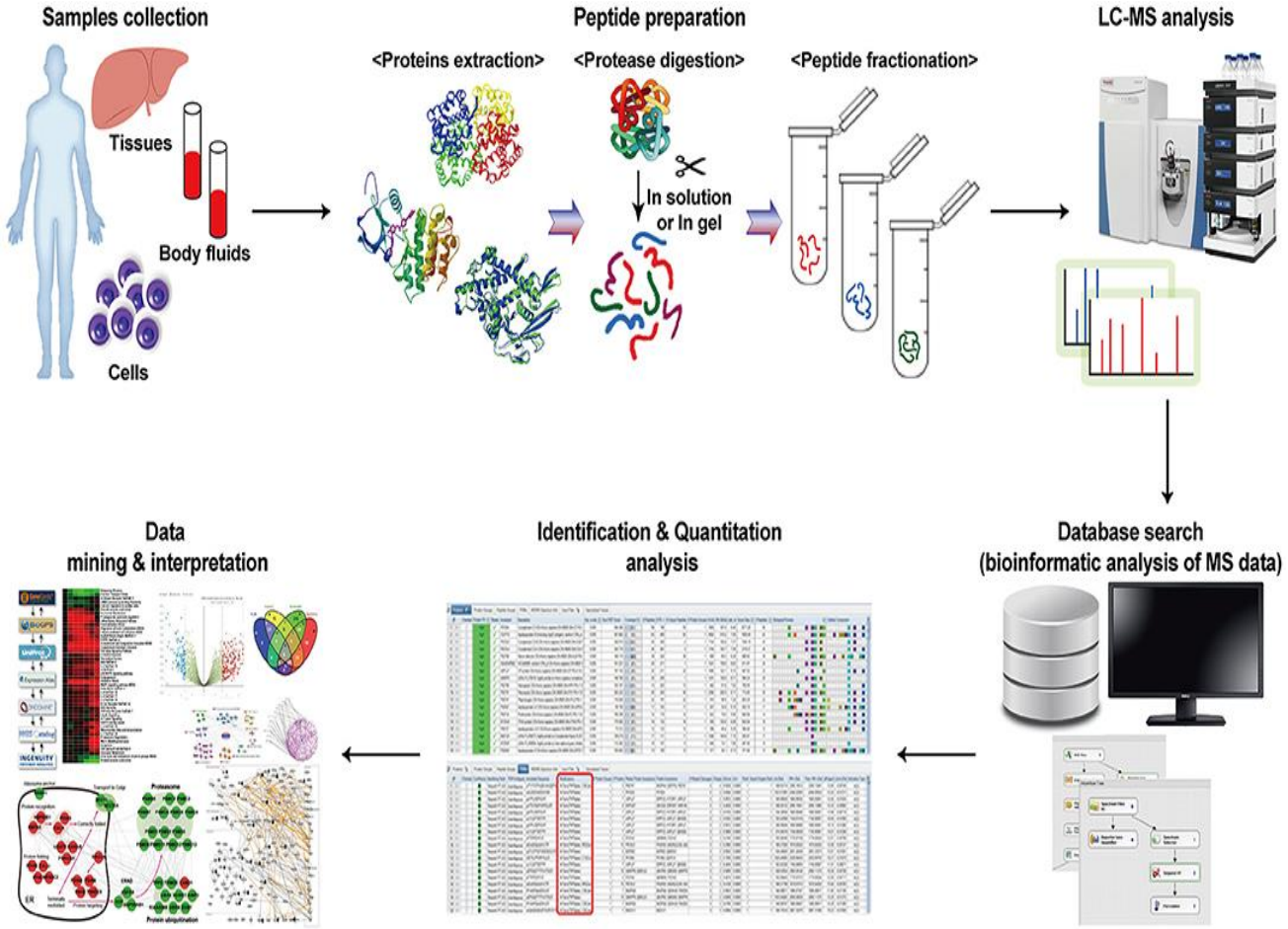
- Demonstrated in metastatic melanoma that spread to the lungs.
- In mice, when antibodies directed against CAMs in the lung endothelium were used as treatment, there was a significant reduction in the number of metastatic sites.



Andreoli, Thomas E. et al. 2013. Molecular Biology of Membrane Transport Disorders

Proteomics

- Proteomics involves a wide range of processes such as protein expression profiling, protein modifications, protein-protein interactions, protein structure, and protein function



Type of Cancer	Sample Type	Method of target discovery	MS-based strategy	Target	Biomarker/ Target Type	Features of biomarker
Liver (HCC)	Patient's tissue	- Proteomics - Phosphoproteomics	In-solution digestion and LC-MS/MS	PYCR2, ADH1A	- Prognostic	HCC metabolic reprogramming
Pancreas	Primary Pancreatic Epithelial cells	- Proteomics	In-solution digestion and LC-MS/MS	LKB1	- Prognostic	Regulate pathways associated with glycolysis, serine metabolism, and DNA methylation
	PDAC cell lines	- Proteomics	In-gel digestion and LC-MS/MS	MAP2	- Prognostic	Proteins involved in microtubule synthesis are upregulated in gemcitabine-resistant cells. Microtubule stabilizing has an effective anti-cancer effect, particularly in MAP2 overexpressed cells.
Ovary	Patients Tissue	- Proteomics	In-solution digestion and LC-MS/MS	NNMT	- Therapeutic	Central metabolic regulator of CAF differentiation and cancer progression in the stroma
Breast	Patients Tissue, Breast cancer cell lines	- Proteomics - Metabolomics	in-solution digestion and LC-MS/MS	PYCR1	- Prognostic	The higher the expression of PYCR1, the lower the patient's survival rate. Expression of PYCR1 is involved in acquiring resistance
	Breast CSCs, Breast cancer cell line	- Proteomics	In-solution digestion and LC-MS/MS	CD66c	- Therapeutic	Proposed as a novel breast CSC marker by modulating the cell viability of CSCs under hypoxic condition.
	Breast cancer cell lines	- Proteomics	In-solution digestion and LC-MS/MS	NEDD4	- Therapeutic	Presenting as a novel therapeutic target by regulating the expression of ALDH1A1 and CD44, which are characteristic of CSCs
Lung	EGFR-mutant cell lines	- Proteomics - Phosphoproteomics	In-solution digestion and LC-MS/MS	PI3K/ MTOR	- Therapeutic	In lung cancer resistant to EGFR tyrosine kinase inhibitor, PI3K/MTOR inhibitor was used in combination to overcome resistance
Myeloid leukemia	Patient-derived AML stem cells	- Proteomics	In-solution digestion and LC-MS/MS	IL3RA, CD99	- Therapeutic	Providing proteomic resources to design leukemic stem cells-targeted therapies by presenting leukemic stem cells-specific markers

AML, acute myeloid leukemia; CAF, cancer-associated fibroblast; CSC, cancer stem cell; EGFR, epidermal growth factor receptor; HCC, hepatocellular carcinoma; chromatography-tandem mass spectrometry; PDAC, pancreatic ductal adenocarcinoma; PDX, patient-derived xenografts.

Type of cancer	Sample type	Biomarker of immunotherapy	Description	Therapeutic monitoring
Liver	Patient's tissue	SLC10A1	Provide predominantly downregulated immune protein cluster between tumor and non-tumor liver	-
Melanoma	Patient's tissue	MHC	Provide linking melanoma metabolism to immunogenicity and immunotherapy	-
Lung	Patient's tissue	LAIR1, TIM3	Identify intratumorally collagen that are major source of immune suppression related to murine and human lung cancer	+
Glioblastoma	Patient's tissue	FAK	Provide glioblastoma factors related to immunotherapy using proteomics/miRNomics	+
Colon	Patient's tissue	IGF2BP3	Provide a novel information of putative tumor-specific biomarkers that are potentially ideal targets for immunotherapy	-
Clear cell renal cell carcinoma	Patient's tissue	OXPHOS, PRDX4, BAP1, STAT1	Provide microenvironment cell signatures, four immune-based clear cell renal cell carcinoma	-
Endometrial carcinoma	Patient's tissue	CDK12	Suggest alternative mechanism for repressing anti-tumor immune response	-

Type of cancer	Sample type	Method of target discovery	MS-based strategy	Target	Biomarker/ target type	Features of biomarker
Glioblastoma	Patient's tissue	- Proteomics	In-solution digestion and LC-MS/MS	YBX1	- Prognostic - Therapeutic	Major tumor invasion-regulated proteins
Glioblastoma	Primary GBM subtypes	- Proteomics	In-solution digestion and LC-MS/MS	CD9	- Therapeutic	Highly expressed in primary GNS cells
Glioblastoma	Glioma cells	- Proteomics	In-gel digestion and LC-MS/MS	EGFRvIII	- Therapeutic	EGFRvIII expression is associated with pro-invasive proteins through EV profile
Glioblastoma	Blood	- Proteomics	In-solution digestion and LC-MS/MS	LRG1, CRP, C9	- Prognostic	Concentration in plasma correlated significantly with tumor size
Glioblastoma	Patient's tissue, Fluid	- Proteomics	In-solution digestion and LC-MS/MS	CCT6A	- Prognostic	CCT6A in EV is associated with induction of expression and amplification and negative survival in glioblastoma
Glioma	Plasma	- Proteomics	In-solution digestion and LC-MS/MS	SDC1	- Diagnostic	High-grade glioma and low-grade glioma through SDC1 present in EV in the patient's plasma
Glioma	Patient's tissue	- Proteomics	In-solution digestion and LC-MS/MS	CDH18	- Prognostic	Role of tumor-suppressor
Astrocytoma	Urine from tumor model	- Proteomics	In-solution digestion and LC-MS/MS	109 proteins	- Prognostic	Protein alteration by date, diagnosis before tumor is seen in MRI

EGFR, epidermal growth factor receptor; EV, extracellular vesicle GBM, glioblastoma multiforme; GNS, GBM-derived neural stem; LC-MS/MS, liquid chromatograp spectrometry; MS/MS, tandem mass spectrometry.

Toxicoproteomics

- Toxicoproteomics is a new scientific method that combines proteomic technologies with bioinformatics.

- Toxicogenomics, a discipline that determines genetic susceptibility of a particular individual following exposure to a carcinogenetic agent, toxicoproteomics allow the monitoring of the body's response to a specific toxicant

- Liver carcinogen N-nitrosomorpholine (NNM) cause, up regulation of stress proteins, including caspase-8 precursor, vimentin, and Rho GDP dissociation inhibitor.

Conclusions

- While basic cell cycle regulators proteins were discovered over 30 years ago, the last decade saw a dramatic increase in our understanding of their role in cancer and their potential as targets for cancer therapy.

- The development of novel compounds using protein target based drug design allowed bringing cell cycle studies from bench to bedside.

- Indeed, development of a CDK4/CDK6-selective inhibitor (Palbociclib) for breast cancer treatment represents the first successful clinical translation in this field.

- Other CDK4/CDK6-selective inhibitors and protein target based drug development have demonstrated encouraging results and their approval is expected in future.

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Thank You