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# INTRINSIC MITOCHONDRIAL APOPTOTIC PATHWAYS; AN IMPERATIVE FEATURE OF CISPLATIN CYTOTOXICTY

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**Keywords:**

Cisplatin, platinum based drug, apoptosis, mitochondrial proteins, cisplatin sensitivity, drug resistance

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**ABSTRACT:** Up till now, the most thriving and extensively used antineoplastic agent with well-established effectiveness is cisplatin. Being a non-specific drug, it reacts not only with genomic DNA but also binds and damages the plasma protein and cytoplasmic protein that confers to cisplatin resistance and severs side effects. DNA damage prompted by cisplatin leads to the activation of many signaling cascades that clue to cell death. This review work comprises interactive pathways of cisplatin with mitochondrial proteins such as p53, p38, p73 and c-Abl which contribute their role in cell death. In addition, disturbance in drug efflux and influx and competent role of repair protein also share in cisplatin resistance. Inhibition of Akt, MKPI, cabl stimulation, amplified activity of p53, p73, and MAPK, increased expression of pro apoptotics (Bax, Bck, Bcd) and decreased expression of anti apoptotic pointers to cisplatin sensitivity. Innovative creation or amendment in a platinum based drug which can magnify the numerous advantageous pathways and precisely constrains the various undesired pathways would greatly contribute to conquest against cancer fight.

**INTRODUCTION:** A metal derived platinum coordination complex cis-diamminedichloroplatinum (II) usually known as cisplatin or cis-DDP was the 1st platinum-based drug introduced in cancer chemotherapy as a DNA damaging agent. Since the discovery of anticancer properties of cisplatin, it has emerged as a most commonly used antineoplastic drug in the treatment of cancer with the well-established effectiveness. It is classified as neutral, inorganic molecule of 11 atoms containing platinum ion surrounded by four ligands Pt $(NH3)_2$ $Cl_2$.

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It has two chloride ion ligands and two ammonia ligands situated adjacent to one another, in a square planar/tetragonal structure. Cisplatin is marketed as Platinol, its IUPAC name; molecular weight and CAS (chemical abstract service) number are dichloroplatinumdiamine, 300.45 and 15663-27-1 respectively.

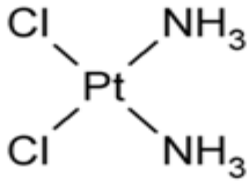


**FIG. 1: STRUCTURE OF CISPLATIN.**

***Cis-Diamminedichloroplatinum2***

## History and chemical nature of Cisplatin:

Cisplatin is a synthetic, cytotoxic drug in the category of alkylating agents. The term

"alkylating" is based on drug's mechanism of action. Basically, alkylating agents are subsequently named so owing to their property of adding alkyl groups to many electronegative groups in cellular environments. Further, these drugs unnecessarily cause the addition of methyl or other alkyl groups onto molecules where they are not essential, resulting in the disruption of DNA function and apoptosis.

However, cisplatin have no alkyl groups and does not prompt alkylating reactions; it has biochemical characteristics parallel to that of bi-functional alkylating agents, so it is appropriately nominated as an alkylating-like drug. Alkylating agents are most active in resting phase of cell so, cisplatin is cell cycle-nonspecific drug.

Cisplatin was 1st synthesized in 1847 by Peyrone but its anticancer properties remain unnoticed till 1960s [1]. In 1965 Dr. Rosenberg discovered this drug while working on effects of electromagnetic field on bacterial cell growth [2]. It was due to cisplatin that a platinum electrode during electrolysis process revealed as an inhibitor of cell division in Escherichia coli, so it was believed that proliferation of fast dividing cancerous cells would also be inhibited by cisplatin [3, 4].

The drug then entered in human clinical trials 30 years ago. These trials culminated in 1978 with FDA approval for the use of cisplatin for the treatment of ovarian and testicular cancer.

Afterwards, cisplatin is being employed in the treatment of epidermoid carcinomas of the head and neck, refractory non-Hodgkin's lymphomas, soft-tissue and osteogenic and Kaposi's sarcoma, retinoblastoma, neuroblastoma, multiple myeloma, melanoma, mesothelioma, gestational trophoblastic tumors, and cancer of uterus, ovary, prostate, urinary bladder, anus, vulva, testis, cervix, lymphatic system, adrenal gland, head, neck, skin, esophagus, thyroid gland, lung (other than small-cell cancer), liver (including hepatoblastoma), breast, stomach, and bile duct. However, it shows great efficacy towards ovarian and testicular cancer.

## Analogues of Cisplatin:

Despite the benefits and success of cisplatin, its consumption is narrow by adverse drug reactions including toxicity pattern, the development of natural and acquired resistance of patients towards the drug, and efficiency against a limited range of cancer. This limited capacity aggravated the searches for functionally and structurally similar alternatives. Search for the new platinum analogues with less deleterious effects have ended up with the emergence of broadly used carboplatin and oxaliplatin.

1.
2.

## Carboplatin

Carboplatin, diammine (1, 1 - cyclobutane-dicarboxylato) platinum (II) was approved in 1989 as a derivative of cisplatin with fewer toxic effects [4]. At present it is the second most extensively used platinum anticancer agent and available as Paraplatin. Carboplatin has a closed cyclobutanedicarboxylate (CBDCA) moiety on its leaving arm in distinction to the readily leaving chloro groups, resulting in better delivery to cells and fewer side effects.

It shared a very parallel activity profile with that of cisplatin and used extensively for ovarian, small cell lung, head, neck and testicular cancers and proved less toxic to kidneys and nervous system. Severe thrombocytopenia is a limiting reaction to carboplatin therapy.

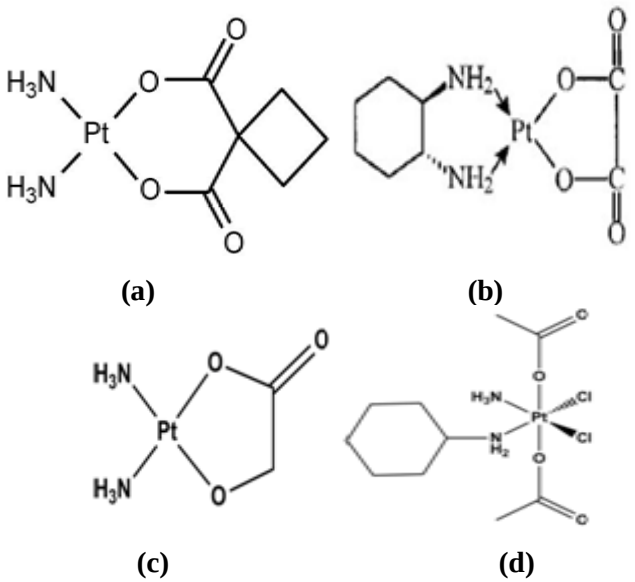


**FIG. 2: ANALOGUES OF CISPLATIN.** *(a) diammine (1, 1-cyclobutane-dicarbooxylato) platinum, (b) [(1R, 2R)-cyclohexane-1,2-diammine] (ethanedioato-O,O' )platinum2, (c) diammine [(hydroxy-κO) acetato(2-)-κO] platinum (d) bis-aceto-ammine-dichloro-cyclohexylamine-platinum (IV)*

**Oxaliplatin**

The third most widely available drug related to cisplatin, is oxaliplatin which is recently approved for colorectal cancer. Marketed as eloxatin, oxaliplatin [(1R, 2R)-cyclohexane-1, 2-diamine] (ethanedioato-O, O') platinum (II) is the only platinum based drug which is active in cisplatin resistant cells [5]. In oxaliplatin, a closed group in the form of oxalate covers the chloro arm of Pt (II), forming a bulkier crosslink that is effective on a different range of cells.

The activity of oxaliplatin has been shown to have a more powerful pharmacological effect than cisplatin. The dose-limiting factor is peripheral neurological toxicity with the absence of nephrotoxicity. Carboplatin is generally less toxic, and, reduced myelosuppression, ototoxicity, nephrotoxicity have been seen. Though, nausea and vomiting remain acute and fight healthy against antiemetics.

**Nedaplatin**

Nedaplatin or diammine [(hydroxy-κO) acetate (2-)-κO] platinum is a platinum-based drug that is manufactured by Shionogi & Co in Japan. It gives the same active constituents as cisplatin inside the cancerous cells upon hydrolysis and show effectiveness in combination therapies [6]. It has been proved to be useful in several solid tumors without renal toxicity. Myelosuppression is reported dose limiting toxicity.

**Satraplatin**

JM216 or bis-aceto – ammine – dichloro - cyclohexylamine-platinum (IV) is the first platinum drug that is administered orally and entered in clinical trial in 1993. Upon metabolism it yields JM118 or cis-amminedichloro (cyclohexylamine) platinum (II) in the blood. Currently satraplatin is in phase III clinical trials to be utilized in hormone refractive prostate cancer and non-small cell lung cancer [7]. Other drugs which are in clinical trials are picoplatin, lipoplatin™ and ProLindac™.

**Side effects of cisplatin treatment:**

All of these analogues (cisplatin, carboplatin, oxaliplatin) are soluble in water and are administered by intravenous injection. Half-life of cisplatin is 20-30 minutes and it is excreted in urine. Within one day, 65-98% of the platinum in the blood plasma is bound to protein. This binding with plasma proteins decreases urinary excretion of platinum and leads to tissue accumulation. Broader use of cisplatin is deterred due to injurious damage to renal system, gastrointestinal tract, nerves, hair follicles, bone marrow and other tissues. Cisplatin treatment results apoptosis in these normal tissues and contributes to severe side effects.

Its side effect includes:

**Nephrotoxicity**: Acute renal failure is the major harmful effect of cisplatin treatment. This renal insufficiency limits the dose that can be given to patient.

**Neurotoxicity:** Dose limiting side effects have been reported by the patients taking cisplatin. Cisplatin therapy causes peripheral neuropathies, those are sensory and reversible but can become irreversible too; out of which sensory neuropathy is the common form that occurs in cisplatin treatment. Several other types of nerve injuries from cisplatin includes; autonomic neuropathies, encephalopathy, myasthenic syndrome, seizures and cortical blindness.

**Ototoxicity**: Deafness and suppressed ability of hearing normal tones is reported during cisplatin chemotherapy. Dose dependent unilateral/bilateral ototoxic effects are more common is children and become severe with repeated dose.

**Hematologic:** Myelosuppression occurs in patients treated with cisplatin. Thrombocytopenia and leucopenia are more observable at higher doses and show their lowest concentrations during treatment. Fever and infection have also been accounted in patients with neutropenia.

Anemia: Hemoglobin level is decreased during the courses of cisplatin therapy. Extreme cautions should be taken while proceeding treatment.

**Ocular:** Blurred vision, cortical blindness, papilledema, optic neuritis, focal deficits, and cerebral blindness have been occasionally seen in cisplatin therapy. However, stoppage of treatment moves towards improvement and total recovery of ocular side effects.

**Hypersensitivity:** Hypersensitivity reactions may include rash, itching, hives, severe allergic reactions, tightness in chest, difficulty breathing, wheezing, tachycardia, facial edema, swelling of the mouth, face, lips and tongue and hypotension within a few minutes of drug administration.

**Serum Electrolyte Disturbances:** Electrolytes disturbance includes hypomagnesaemia, hyponatremia, hypocalcemia, and hypophosphatemia which cause various problems with cell operation in patients treated with cisplatin.

**Others:** Blood in urine, loss of balance, decrease of blood cells in bone marrow, frequent diarrhea, changes in food tastes, extreme fatigue, numbness in the extremities along with general side effects of chemotherapy.

**Cisplatin in combination therapies:**

Cisplatin can be given in combination with radiation therapy and numerous anticancer cytotoxic agents such as taxanes and gemcitabine to get the maximum effects. It is used in combination with many agents in various regimes. Combinations of these agents have been reported to have better response rates than the cisplatin alone.

Cisplatin is generally used in following combination therapies: 1.

Vinblastine, Bleomycin and Actinomycin D in testicular cancer

Cyclophosphamide, Doxorubicin and 5-Fluorouracil in ovarian cancer

Methotrexate and also Bleomycin in head and neck cancer

For instance, Gemcitabine/Cisplatin/S-1(GCS) combination therapy is used in biliary tract cancer and metastatic urothelial carcinoma [8, 9]. Bleomycin, Etoposide and Cisplatin (BEC) combination therapy is effective in ovarian granulosa tumor cells [10]. Paclitaxel gives best effects in cisplatin resistant cells [11]. Anti–4-1BB and cisplatin are also used in tumor killing and prevention of organ-specific toxicity [12].

Moreover, some non cytotoxic agent such as nifedipine enhances the effects of cisplatin [13]. Cisplatin given in combination with calcitriol may be effective multidrug therapy option in the treatment of high-risk retinoblastoma [14]. Fisetin is a flavonoid found in fruits and vegetable that inhibits propagation of numerous types of cancer cells. Fisetin and cisplatin together activate intrinsic mitochondrial apoptotic pathways which could be effectively employed in combination to eradicate embryonal carcinoma cells [15].

Promising effects were observed by using salvage regimen of oxaliplatin and cisplatin in treating cisplatin-refractory germ cells tumors [16]. TIP regime consisting of taxol, ifosfamide and cisplatin provoked momentous anticancer effects in cervical cancer and head and neck cancers of squamous cells [17]. The use of nimotuzumab along with cisplatin and 5-fluorouracil is safe and noteworthy in advanced esophageal squamous cell carcinoma [18].

**Target actions of Cisplatin:**

Cisplatin forms covalent adduct with many biological molecules, but its principle target is DNA. It acts like a cross-linking agent and attacks the activity of cancer cells by binding to DNA and starts its cytotoxic effects. The initial steps of eliciting apoptosis by platinum compounds include four stages which are:

Cellular accumulation by passive and active uptake with the involvement of few transporters which are:

- Canalicular multi specific organic anion transporter 2 &1, multidrug resistance-associated protein 1, 5&6, solute carrier family 22 member 2, high affinity copper uptake protein 1&2 and copper-transporting ATPase 2 &1 [19].
- Activation of the platinum complex
- Binding to nucleic acids to give rise multiple of Pt-DNA adducts i.e., inter or intra-strand DNA cross-links
- The cellular response to DNA damage.

The DNA damage affects numerous cellular courses together with transcription and replication. After formation of cisplatin induced DNA adducts, the Pt lesions are either cleared by nucleotide excision repair (NER) or cell death is induced by many DNA damaging recognition proteins. But not all platinum complexes indisputably cause cytotoxicity. For instance, the trans-isomer of

cisplatin is not a good chemotherapeutic. Different arrangement of ligands in both these isomers, results in different binding manner with DNA. Trans cis-DDP produces DNA-histone and histone-histone cross links. Its little activity is usually due to its fast degradation before binding with DNA [20].

**Cell entry of Cisplatin and role in Influx/Efflux:**
The very first step concomitant with platinum-containing compounds is the drug uptake that comprises its influx and efflux from cells by transporters and chaperones which are involved in the Cu homeostasis in cells. The significant proteins which are involved in the cisplatin influx are; Copper transporter 1&2, the chaperone Atox1. CTR1 mediates buildup of the Pt-containing drugs; on the other hand CTR2 decreases their influx [19].

High concentration of copper and cis-DDP has been shown to trigger the degradation of CTR1, which is assisted by Atox1 and hence it also contributes in down regulation of its influx transporter [21]. So, cells resistant to cisplatin are cross-resistant to Cu and vice versa. The copper transporters ATP7A, ATP7B regulates efflux of cis-DDP **(Fig. 3)**.

Reduced drug accumulation is related to ABC transporters that includes MDR genes (MDR1, MRP1, MRP2 and LRP). Recently, MRP2, the canalicular multi specific organic anion transporter (cMOAT) has been characterized and found to be over expressed in a number of cisplatin resistant cells. However, any change in the expression of these proteins leads to the altered sensitivity of cytotoxic effect of Pt containing drugs [22, 23].

**Access to core target; the DNA:**
After drug entry, the main event accountable for the cisplatin cytotoxicity is binding with DNA. Cisplatin contains two chloro ligands **(Fig. 1)** that stay connected with platinum due to normal chloride concentration in the plasma. After the drug uptake, the abrupt decrease in chloride concentration causes cisplatin to endure aquation (i.e. The chloro ligands are substituted by water ligands) which in turns activates the cis-DDP [Pt $(NH_3)_2Cl(H_2O)]^+$ and $[Pt(NH_3)_2(H_2O)_2]^{2+}$. Once inside the cell, cisplatin has a number of potential targets: like proteins, membrane phospholipids, DNA, RNA, sulfur-containing enzymes such as metallothionein and glutathione; and mitochondria. Foremost target leading to apoptosis is the genomic DNA **(Fig. 3)**.

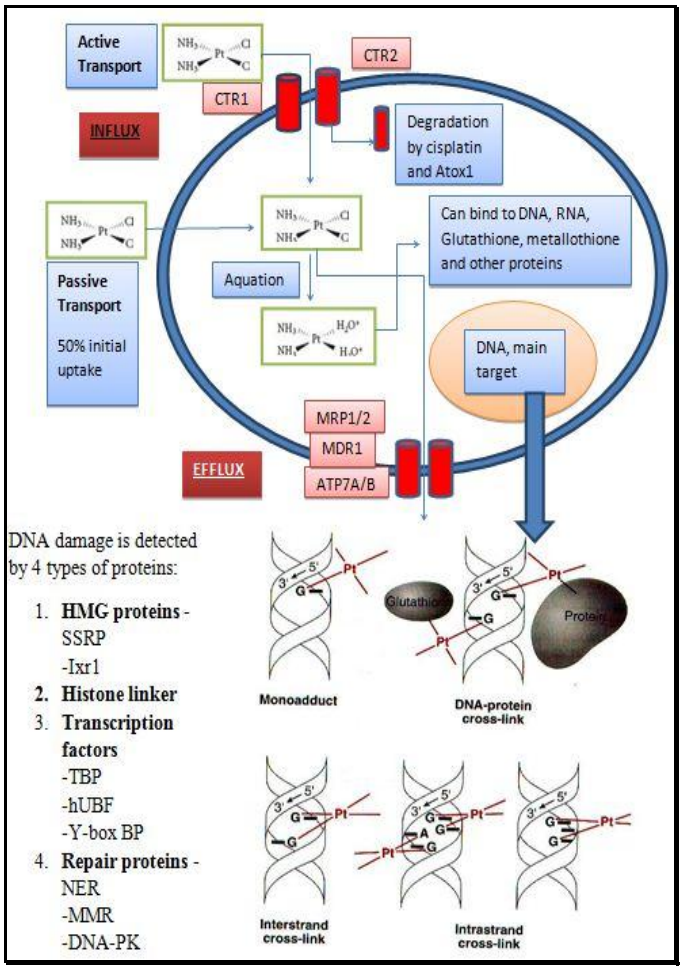


**FIG. 3: DRUG ENTRY, TARGET AN ACTION SITE, FORMATION OF DNA ADDUCTS AND DAMAGE RECOGNITION PROTEINS**.
Copper transporter 1 mediates accumulation of cisplatin and CTR2 limits its influx in cell. The copper transporters ATP7A, ATP7B regulates efflux of cis-DDP. Upon entrance cisplatin binds with its main target i.e., DNA to form DNA adducts which are detected by damage detector proteins such as linker histones H, repair-related proteins, HMG box-containing proteins and transcription factors.

Of the many prospective sites available, cisplatin selectively binds with the N7 of Adenine or Guanine. 90% 1,2-intrastrand cross-links are formed, of which 65% are to two adjacent $N^7$-Guanine sites [5'-d(GG)] and 25% are to adjacent $N^7$-Guanine and $N^7$-Adenine sites [5'-d (AG)]. The others are intra-strand cross-links, inter-strand cross-links, mono-functional adducts and protein-DNA cross-links [24, 25].

Formation of these adducts unwinds the DNA and twists it toward the major groove and the minor groove becomes wide and shallow, hindering transcription and replication activity and thus initiates cellular repair mechanisms which ultimately leads to apoptosis. In the beginning, an effort to recoup DNA is effected by excision or mismatch- repair mechanisms, which confiscates the damaged DNA part. The altered structures by platinum adduct, such as bending and unwinding of DNA, appeal various damaged DNA binding proteins **(Fig. 3)**.

Binding of these proteins are thought to potentiate the anticancer activities of the platinum drugs. Four classes of damage detector proteins have been identified including linker histones H [26], repair-related proteins, HMG box-containing proteins (Structure specific recognition protein 1 SSRP1, Ixr1) [27], and transcription factors (TATA binding protein TBP, human upstream binding factor hUBF, Y-box binding protein) that recognize and bind selectively to *cis*-DDP-modified DNA [28].

DNA repair nonetheless, does not arise at a considerable rate for fast dividing cells; thus, the existence of malfunctioning DNA is detected by cell cycle check-points, and prompts a sequence of reactions that lead to cell death. If the rates of DNA platination go beyond the rate at which these adducts are detached by repair, cells nearly go to death and expire.

**Signal transduction as an imperative feature of cisplatin cytotoxicity:**

**Instigation of ATM/ATR:**

DNA adducts formed by cisplatin are sensed by main regulators of DNA damage response, which are phosphatydlinositol kinase-like serine/threonine protein kinase ATM (ataxia talangestica mutated) and ATR (ataxia talangestica mutated Rad-3 related). Both these are actuated by auto-phosphorylation and are involved in the DNA repair, apoptosis and cell cycle arrest. ATM/ATR then activates many downstream substrates i.e., Chk1, Chk2 and p53 kinases by direct phosphorylation **(Fig. 4)**. ATM is predominantly activated by the double strand DNA breaks along with the activation of Chk1 which prevents tumor advancement. ATR functions downstream of ATM in reaction to DNA damage but are also activated autonomously of ATM. ATR retort to DNA lesions by ATR-Chk1 pathway. Inhibition of ATR activity has been shown in regulating cisplatin sensitivity [29].

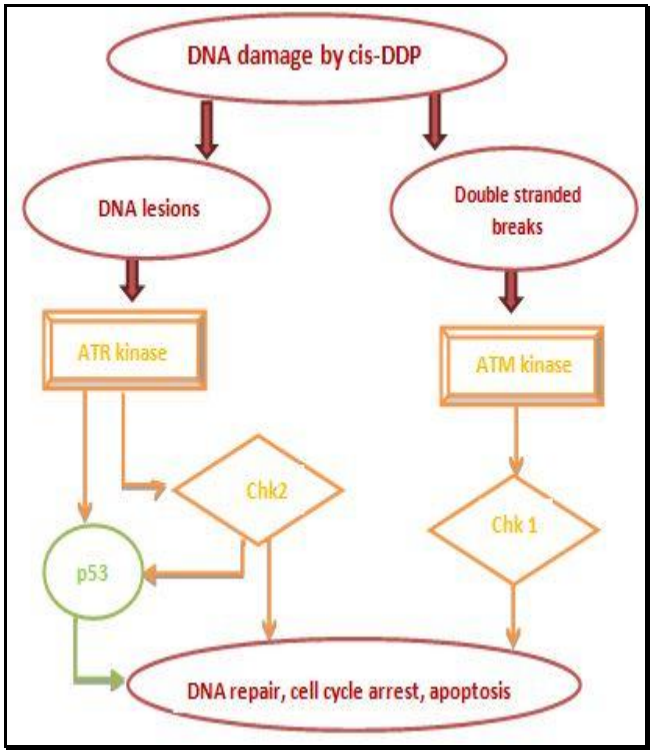


**FIG. 4: ACTIVATION OF ATM/ATR**. ATM and ATR sense DNA adducts formed by cisplatin and activate many downstream substrates which are engaged in DNA repair, apoptosis and cell cycle arrest.

**Cell cycle arrest (G1 and G2 arrest):**

The cell cycle is divided into 4 stages; G1 (Gap phase1), S (DNA synthesis phase), G2 (Gap phase 2) and the last one M (mitosis phase). DNA damage induced by chemotherapeutic agents (i.e., cisplatin) leads to the activation of cell cycle check points at different stages; these comprise G1/S checkpoint, S- phase arrest and G2/M arrest due to the suppression of Cdc2-cyclin A or B kinase by p53 dependent and independent manner **(Fig. 5)**.

Cdc2/Cdk1 and its regulator cyclin B are vital mediators of G2 phase of mitosis. DNA damage check point proteins ATM/ATR activates upon cisplatin exposure to DNA, and subsequently Chk1 and Chk2 are activated [30].

In case of double stranded breaks formed by DNA adducts, Chk1 is activated as a downstream of ATM and prevents the activation of Cdk1 by Cdc25. Chk1 phosphorylates and inactivates

Cdc25A and Cdc25C which is then arrested in the cytoplasm by 14-3-3 proteins, thereby resulting in activation of Cdk1/cyclin b and succeeding G2 arrest. 14-3-3 is up-regulated in p53 dependent manner in response to ATM/ATR and maintains G2 arrest. On the other hand, ATR activates Chk2 and p53 that leads to the activation of p21 and fallouts in the G1 growth arrest or apoptosis **(Fig. 5)**. Studies have shown that inhibition of ATR as compared to Chk1 inhibition, in cancer cells makes them sensitive to cisplatin and to its analogues [31].

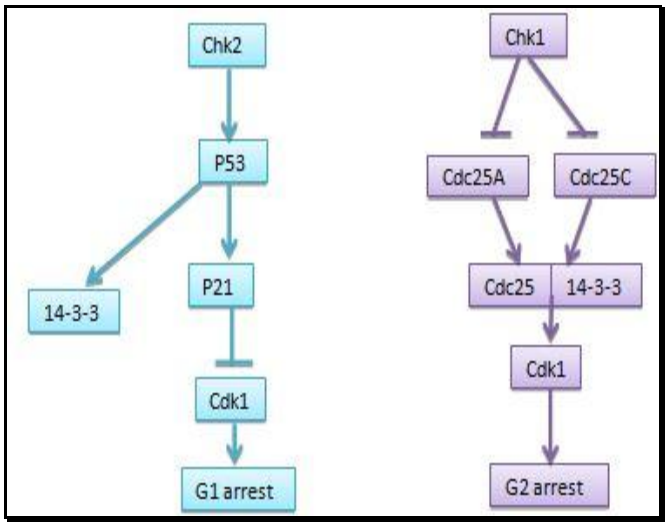


**FIG. 5: CISPLATIN INDUCED G1 AND G2 ARREST**. Chk1 is activated as a downstream of ATM and causes G2 arrest by inhibiting Cdc25A and Cdc25C. ATR activates Chk2 and p53 causing activation of p21 and inhibiting Cdk1 leading to G1 arrest.

**Intrinsic mitochondrial pathways leading to apoptosis or cell survival:**

**Potential role of p53:**

P53, tumor suppressor protein is a key downstream effector of all of these DNA damage kinase pathways and is a chief component of intrinsic mitochondrial pathway. This pathway ends up with apoptosis by the aid of many proapoptotics and release of cytochrome C due to the signals initiated from cell vicinity. P53 is known as the custodian of the genome which is turned on in response to DNA damage, resulting in inhibition of cell cycle and DNA repair or apoptosis. P53 regulates apoptosis by the transcriptional activation of many proapoptotic proteins that includes Fas, DR4& 5, Bax, Bid, PUMA and NOXA or by direct activation of Bax or Bak **(Fig. 6)**. HMG1/2 facilitates the binding of p53 to DNA and stimulates its trans-activation. P53 is a short timed protein that is degraded by ubiquitin degrading pathway. P53-dependent signaling in normal cells, results in cell cycle arrest by p21, if the DNA damage is inclusive, then p53-dependent pathways ends up with the death of damaged cell [32].

Cells lacking functional p53 are challenging to cisplatin but after introduction of p53, they become sensitize. Absence of phosphatase and tensin homologue (PTEN) expression which blocks the activity of Akt or the augmented mouse double minute 2 (MDM2) expressions are allied with the decreased activity of p53 in tumor cells.

MDM2 boosts tumorigenic potential and acts as negative feedback regulator of p53 that maintains its stability and is up regulated by p53 itself. P53 initiates mitochondria-independent apoptosis by promoting activation of Fas/FasL which further activates caspase 8 and caspase 3 that ends up with apoptotic cell death in cisplatin sensitive cells **(Fig. 6)**. However, Fas/FasL activation essentially does not necessitate only p53 dependent stimulation [33, 34].

P53 has transcription-dependent and transcription-independent pro-apoptotic activities. Its transcriptional role provokes the genes encoding BH3-only members of the Bcl-2 family, NOXA and PUMA, which inhibits the functions of anti-apoptotic Bcl-2 or Bcl-XL by promoting Bax/Bak activation. The transcription-independent activity of p53 includes the direct stimulation of pro-apoptotic Bak or Bax, inhibition of anti-apoptotic Bcl2 or Bcl-xl and prompting the mitochondrial outer membrane permeabilization, eventually leading to apoptosis [35].

In addition to role of p53 in apoptosis, it also tempt cell-cycle arrest by the activation of CDKN1A gene which codes p21 (WAF1 or Cip1). P21 gene is induced after DNA damage and its induction is necessary for the p53-dependent cell-cycle arrest after DNA damage. It then inhibits CDK2 that fallouts in cell survival **(Fig. 6)**. So, p53 has dual role in response to cisplatin induced DNA damage that is either cell death or DNA repair [36]. Another important downstream target of p53 is GADD (growth arrest and DNA damage 45 gene) that is

activated by wild type p53 during cisplatin tempted DNA damage. It can interrelate with p21 and PCNA (proliferating cell nuclear antigen) ensuing cell cycle arrest and cell survival **(Fig. 6)**. It has also been shown to contribute in DNA repair system by cooperating with NER and shelters cell from cisplatin induced cytotoxicity. Hence, gadd45 gene may be an important negotiator of the anti oncogenic action of p53 consequential DNA damage [37].

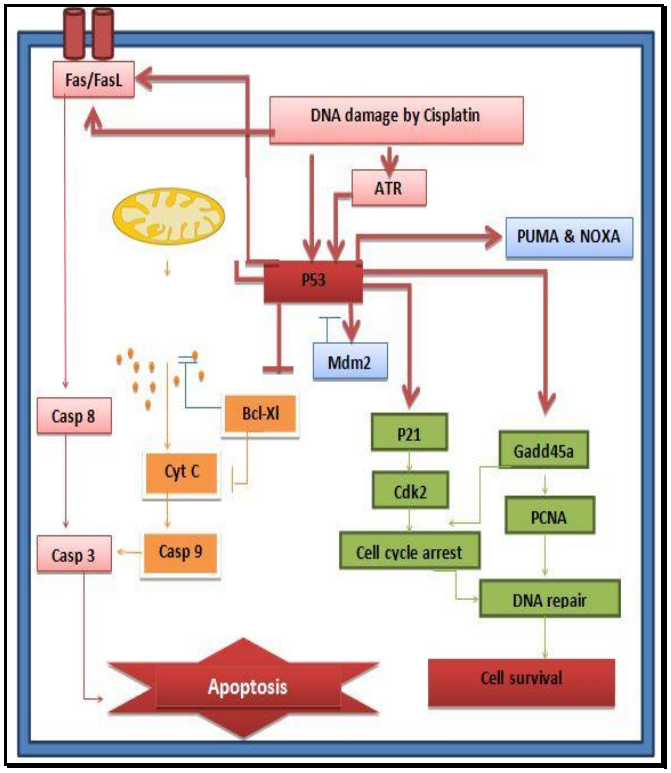


**FIG. 6: ROLE OF p53 IN CIS-DDP CYTOTOXICITY.** p53 initiates mitochondria-independent apoptosis by promoting activation of Fas/FasL which further activates caspase 8 and caspase 3 that ends up with apoptotic cell death in cisplatin sensitive cells. p53 is involved in regulation of apoptosis by transcriptional activation of many proapoptotic proteins such as Fas, DR4& 5, Bax, Bid, PUMA and NOXA or by direct activation of Bax or Bak. p53 also targets GADD which excites proliferating cell nuclear antigen resulting in cell death.

## c-Abl and cisplatin:

The non-receptor tyrosine kinase, c-abl gene is involved in the regulation of many cellular processes including proliferation, differentiation and cell death. DNA damaging agents such as cisplatin contributes to the activation of c-abl gene that contributes in the down regulation of Cdk2 and p53 dependent G1 arrest. Though, the expression of this proto oncogene is suppressed by the action of RB (retinoblastoma) and p53 **(Fig. 7)**.

RB is a growth inhibitor which inactivates many growth regulators and its principle target is c-abl [38]. In addition to the p21 independent and p53 dependent growth arrest by c-abl, it also activates p38 MPK and SAPK/JNK. Acting as upstream regulator of MEKK1 and successive activation of JNK, this pathway leads to the commencement of apoptosis in response to cisplatin. On the other hand, p38 MAPK and SAPK/JNK activation is not only dependent on c-Abl, cisplatin also directly and indirectly activates these death inducing signaling cascades [39].

Some studies have shown that, cisplatin persuaded activation of c-abl also steadies the p53 homologue p73, a p53-related proapoptotic transcription factor which ultimately ends with the cell death by apoptosis. Cancer cells become extremely resistant to cisplatin cytotoxicity in the absence of c-Abl and p53. So, c-abl increases the apoptosis inducing effect of p73 in the presence of wild type p53 and also in p53 deficient cells.

C-abl phosphorylates widely expressed ΔNp63$\alpha$ isoform of p63, which induces the binding of Yap resulting in its stability **(Fig. 7)**. In addition to this, c-abl also phosphorylates Tap73 in response to cisplatin and hence promotes its cytotoxicity by increasing the levels of pro apoptotics [40].

## p73; Isoform of p53:

P53 is commonly mutated in many types of cancer that correlates with cisplatin resistance. Another homologue of p53 is p73, which plays an important role in cisplatin cytotoxicity in the absence of wild type p53. Like p53, p73 regulates the transcription of many downstream genes and also participates in cell cycle arrest by the trans-activation of p21 in cancer cells, thus compensates the deficiency of p53. Interestingly, isoforms of p53 are structurally quite similar and are activated upon same stress signals, but their upstream regulators are strictly different from each other as well as their inhibitory proteins are also dissimilar; p53 is suppressed by Mdm2 and p73 is from ITCH **(Fig. 7)**. P73 acts as a downstream target of various signaling cascades including MAPK & c-abl, both MAPK (i.e., JNK)

& c-abl activates p73 and subsequently initiates apoptosis upon DNA damage. Cancer cells without miss match repair system and c-Abl are more prone to cisplatin resistance. This is due to the inactivation of p73 by c-Abl, both are associated with cisplatin induced cytotoxicity and are important parts of mismatch-repair-dependent apoptosis pathway. Cisplatin increases the half-life of p73 by its direct activation and c-abl dependent activation [41].

Another important protein that is activated by p73 in response to cisplatin provoked DNA damage is Yap (Yes-associated protein) that binds and excites the transcription of many pro apoptotic genes **(Fig. 7)**. YAP also stabilizes p73 by reducing the interaction of ITCH with p73. However, Akt negatively regulates the interface between YAP and p73, thus subsidizes in cisplatin resistance.

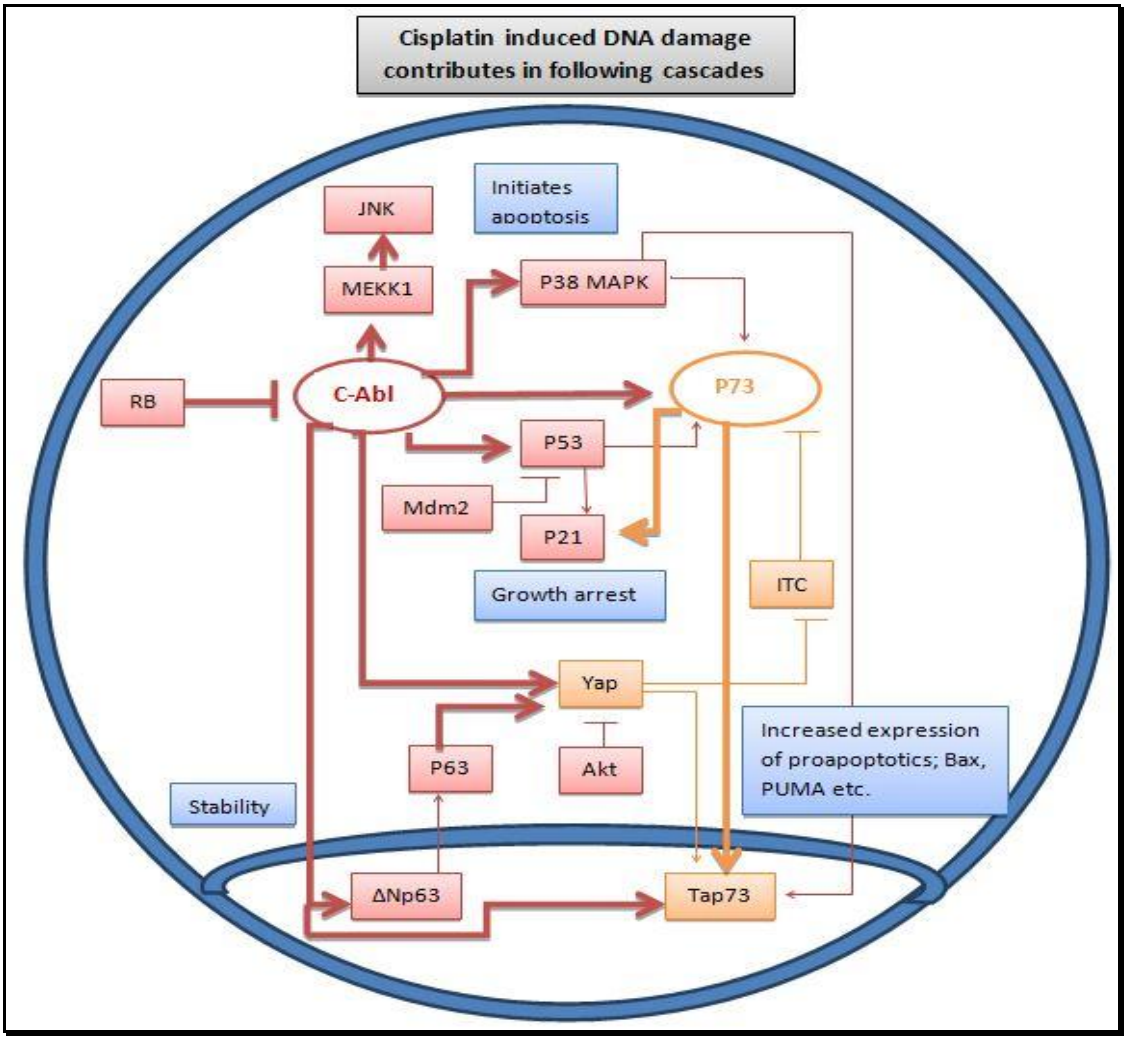


**FIG. 7: c-ABL AND p73 CROSSTALK INDUCED BY CISPLATIN**. Cisplatin induced c-Abl and p73 downstream pathways are shown. Cisplatin contributes to c-Abl gene activation which causes p53 dependent growth arrest. Another homologue of p53 is p73 which arrests cell cycle by trans-activating p21 in the presence of cisplatin. P73 also induces yes-associated protein that promotes many pro-apoptotic genes.

## CONCLUSION AND PERSPECTIVES:

Platinum compounds are important therapeutic agents in the world of cancer which exerts their advantageous and anti cancerous effects in epithelial malignancies (head and neck, ovarian,

lung etc.) also in advance/metastatic malignancies (prostate, breast, pancreatic and melanomas etc.). Cisplatin and other analogues such as carboplatin and oxaliplatin, attacks on DNA, form DNA adducts and initiates signal transduction of major apoptotic and survival proteins that ends up with cell death in both cancer cells and as well as in normal cells. This effect of cisplatin may also appear in the form of severe side effects that is the major limitation of cisplatin chemotherapy.

Cisplatin principally works as a general cytotoxic agent but in order to make it more influential antineoplastic agent, targeted therapies should be practiced that may spare the normal tissues.

Researchers should focus on forming a novel platinum compound that can form only DNA intrastrand cross link which is ultimately accountable for activation of apoptosis. At molecular level, another aspect is the dissimilar behavior of different tumors against cisplatin that fallouts in the different pattern of signal transduction, activation of transcription factors and enzyme activities which increase the chemo resistance. Understanding these signaling pathways that intensifies the drug resistance can expose new trends for pharmacological manipulation of cancer chemotherapy with cisplatin. Researchers should emphasis on the development of tumor specific platinum complexes that can accumulate only in specific tumor cells; this would expand the capability of platinum drugs to treat cancer.

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