Dean&Francis
ISSN 2959-409X

Evolving Resistance: The Interplay of p53, Chemoresistance, and Metastasis in Cancer's Journey

Zenith Kwong[1]

[1]Li Po Chun United World College of Hong Kong, Hong Kong, China
*Corresponding author: Zenith26@lpcuwc.edu.hk

Abstract:

Cancer is a growing concern around the world with more than 20 million new cases confirmed and counting. This is partly caused by the overuse of Chemotherapeutic drugs against Cancer, leading to its potency. Cancer starts with an accumulation of genetic mutations in the genome, and the ignorance to cell checks by CD8 and NK cells. The Cancer cell utilizes its hallmark capabilities and uses one of its cells to metastasizes in other areas of the body either through the perineural, circulatory, or lymphatic system. p53 is a tumor suppressor gene which repairs the mutations of the cell by conducting cell cycle arrest and inducing apoptosis. However, cancer cells can increase the expression of SB100 which can inhibit p53. One solution is to combine PRIMA-1 and CDDP which restores p53 and it's wild type functions to induce apoptosis. This effect is increased with the use of adenoviral DN-Akt since it could increase p53 phosphorylation at Ser15.

Keywords: Cancer, Metastasis, p53, Cisplatin, DN-Akt,

1. Introduction

The issue of Cancer has received considerable critical attention. According to the International Agency for Research on Cancer (IARC). There were close to 20 million new cases of cancer in the year 2022 (including nonmelanoma skin cancers [NMSCs]) alongside 9.7 million deaths from cancer (including NMSC), estimating that 1 in 5 men or women develop the disease. Among which, Lung Cancer was the most frequently diagnosed at 2.5 million cases totaling to 12.4% of the percentage [1]. Enter p53, a tumor suppressor gene, have been susceptible to mutations, essentially lowering its effectiveness. With the leading causes of cancer being Obesity and Tabacco, contributing up to 85% of lung cancer [1] and 20% of all cancer types [2] respectively. Moreover, the overuse of chemotherapeutic drugs has caused chemoresistance lowering these drugs' overall effectiveness. The accumulation of gene mutations has caused normal cells to become cancerous cells. Many therapeutic solutions have been developed since its first discovery in 3000BCE.

2. The Life Cycle of Cancer

2.1 How Cancer Starts

As shown in figure 1, the life of Cancer starts with

an accumulation of genetic and epigenetic mutations in normal cells [2,3], resulting in alterations of the genome. These alterations allow the cell to cancel or ignore normal cellular processes such as Apoptosis, ignoring cell check by CD8 or NK cells and acquiring hallmark capabilities that aid in cancer growth [2,3]. This would normally be regulated by suppression factors and pathways such as p53 or RB pathways. However, due to the sheer usage of these two pathways in the genome, they are highly likely to be mutated due to their need to loosen when transcription, becoming more exposed to its surroundings [4,5,6,7]. Without these suppressors, the genome becomes unstable and is filled with mutations. Leading to dysregulated cell signaling and unregulated growth control [2,3,6,7]. This essentially allows for the tumor to modify the chromatin structure of the tumor to be modified without altering its DNA structure [2]. This is possible due to its structure being in a tightly packed(heterochromatin) or more relaxed state(euchromatin) which dictates it's gene accessibility [8]. The tumor uses this to manipulate the structure of the chromatin through DNA methylation and histone modifications of multiple molecules released by the tumor. One such groups are DNA Methyltransferases, which add Methyl groups to the DNA, which silences genes [8]. Another such groups are Histone Modifying Enzymes which add or remove chemical groups to histone proteins, the basic unit of chromatin packaging, to control how tightly DNA is wrapped around histones. One such example of this is the SETD18, which add methyl groups to histones, and histone deacetylases (HDACs), which remove acetyl groups [8]. Aside from these molecules, this phenomenon can also be activated by signaling molecules involved in cellular communication. For example, Growth factors such as TGF-β1 can activate downstream signaling pathways, such as CCR7 that converge on epigenetic regulators, altering gene expression patterns [4,6,9].

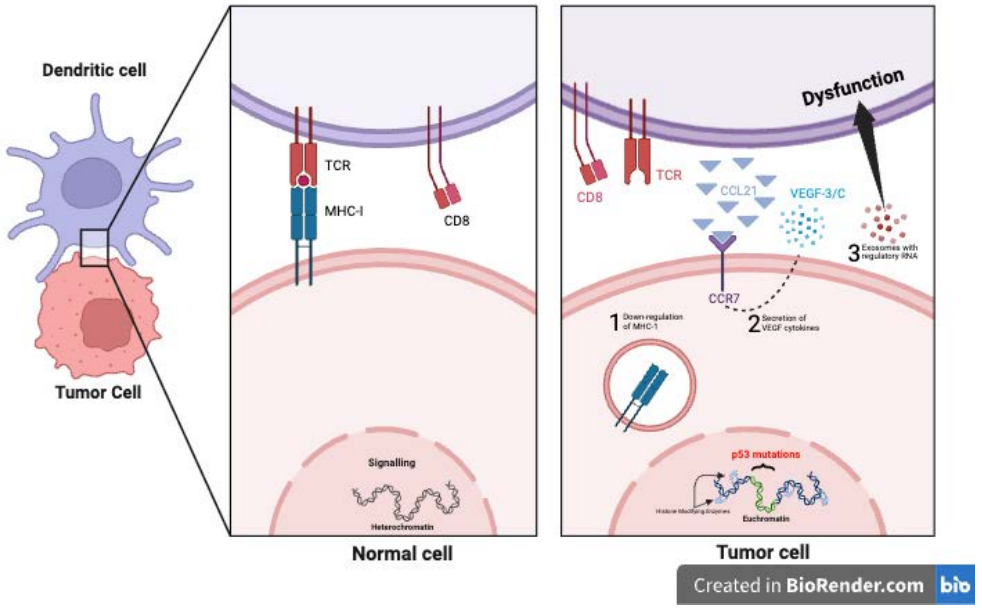

Figure 1. Comparison of the Cell Check process towards an unmutated cell against a cancer cell

2.2 Metastasis

Metastasis involves a series of steps, including invasion, intravasation, extravasation, and colonization. It acts as a checkpoint and a critical turning point for the progression of cancer [9,10].

2.2.1 Invasion

This process starts with the downregulation of cell adhesion molecules, such as E-cadherin, a protein which cells use to stick to one another, which allows the tumor to escape the primary mass of the tumor and become mobile [10].

At this stage, for them to become more invasive, they undergo a series of changes which make them go more mesenchymal like, allowing them to become more migratory and invasive. This process is usually controlled by transcription factors such as, ZEB1, SNAIL1, TWIST, and SOX family members to control this transition through

ISSN 2959-409X

the downregulation of epithelial genes and mesenchymal genes [6]. These transcription factors are crucial for the maintenance of the epithelial-mesenchymal transition (EMT) phenotypes (ZEB1) but also to prevent the disruption of suppressor genes such as p53 or mdm2(TWIST) [6,8,9]. One notable factor is the contribution of EMT and TGF-β1, which aid in the acquisition of stem-cell-like properties and invasive characteristics [9].

As seen in figure 2, for cells to create a path for invasion, the cell must first degrade the ECM. The ECM is a complex network of proteins and other molecules that provides structural support and regulates cellular behavior [10]. They first do this by secreting Matrix Metalloproteinases(MMP), a family of zinc-dependent endopeptidases into the extracellular space such as MMP1/Collegenase-1, serine proteins and more to degrade components in the ECM such as collagen, fibronectin, proteoglycans. Since they are injected in an inactive form, they need to be activated. Alternatively, they can be activated by the ECM though various growth factors, proteases and inflammatory mediators. Once activated, MMPs bind to their specific ECM components and cleave them, disrupting the integrity of the matrix. For example, MMP1, targets and degrades collagen, which is the most abundant protein in the ECM. Once the ECM is unstable enough, the tumor cells then remodel the ECM to better fit the tumor, creating a tumor promoting environment by making gaps or pathways through which cancer cells can migrate to.

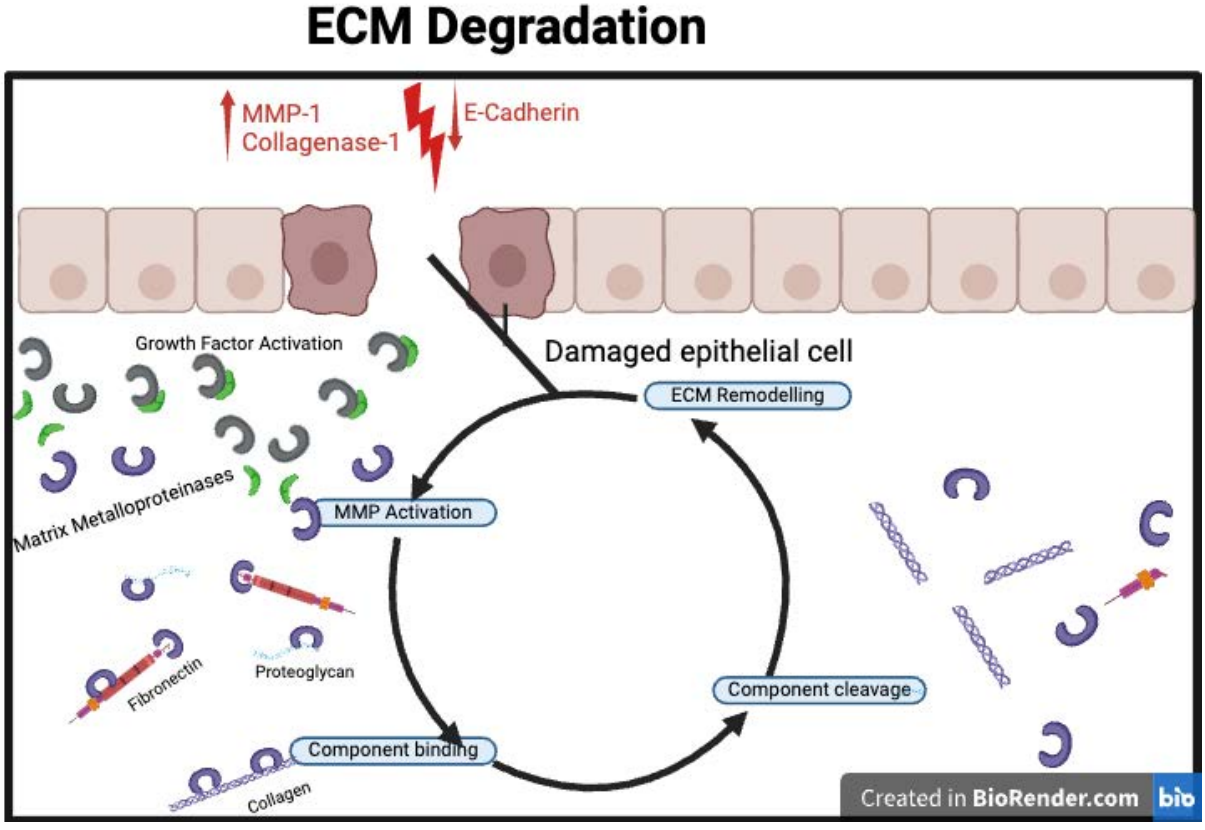

Figure 2. Cycle and Process of ECM Degradation

2.2.1 Intravasation

From this point, the tumor cell has multiple locations to spread to as they can enter the lymphatic, circulatory or nervous system. Below are their methods of spreading in more detail.

2.2.1 .1 Perineural Spread

PNI is associated with increased risks of local recurrence, regional metastasis, and ultimately, poorer patient prognosis [11,12]. While the precise mechanisms orchestrating PNI remain elusive, research highlights a complex interplay of tumor cell behavior and their interaction with the nerve microenvironment [12]. Tumor cells demonstrate an affinity for the neural environment, exhibiting neurotrophic characteristics that enable them to modify and invade nerve structures. This invasion process involves a series of steps that facilitate tumor cell adhesion, penetration, and migration along nerve pathways.

Tumor cells express neurotrophic growth factor receptors, notably tyrosine receptor kinase A, B, and C, which

respond to nerve-derived growth factors, guiding their migration and enhancing their survival within the perineural space [11,12]. The expression of nerve cell adhesion molecule (N-CAM) on tumor cells further aids their attachment to nerve structures, allowing them to breach the perineural sheath and initiate invasion [11,12].

The deposition of laminin-5, a crucial component of the basement membrane, is also implicated in PNI. Laminin-5 modulates cellular adhesion and participates in inflammatory signaling within the nerve microenvironment, creating a favorable environment for tumor cell invasion [11,12]. Additionally, tumor cells exploit their capacity to secrete matrix metalloproteinases (MMPs) to degrade the perineurium and endoneurium, the protective sheaths encasing nerve fascicles. This enzymatic degradation weakens the nerve's structural integrity, providing routes for tumor cell penetration [11,13].

Once inside the perineural space, tumor cells can migrate along the nerve, using it as a conduit for growth and dissemination [14]. Histological examination of PNI reveals prognostic indicators that reflect the severity of invasion. The diameter or largest nerves involved, particularly those with a diameter of 0.2 mm or greater, is associated with a higher risk of adverse outcomes [12]. Similarly, the involvement of multiple nerves within a histological field point to extensive PNI and a greater risk of progression [12].

2.2.1 .2 Lymphatic and Circulatory Spread

After migrating to the walls of the lymphatic vessels through the following of chemotactic signals, the tumor cells encounter the lymphatic endothelial cells [9,10]. Tumor cells expressing the chemokine receptor CCR7 are attracted to the lymphatic endothelial cells that secrete the chemokine CCL21. This interaction guides the directed migration of cancer cells into the lymphatic system, similar to the trafficking of dendritic cells during inflammation [9,10]. These triggers signaling pathways which promote the loosening of the endothelial junctions between cells, which downregulates adhesion molecules such as E-Cadherin, to create gaps in between the cells for the tumor to slip between. Tumor cells then secrete Vascular endothelial growth factor – C(VEGF-C) [9]. This simulates lymphatic sprouting from pre-existing lymphatic vessels toward the tumor, increasing the density of lymphatic vessels near the tumor and increasing the density of lymphatic vessels near the tumor and creating new entry points for tumor cells [9,10]. VEGF-C can also induce the dilation of lymphatic vessels, potentially making them more permeable and facilitating tumor cell entry [10]. Tumor cells disrupt the tight junctions by secreting proteolytic enzymes like MMPs, which degrade junctional proteins, and by exerting mechanical force on the lymphatic endothelium to widen the gaps between cells. Breaching the basement membrane, a key step for tumor cell entry into the lymphatic vessel lumen, involves the secretion of MMPs that target and degrade type IV collagen, a major component of the basement membrane. Tumor cells may also extend membrane protrusions called invadopodium, which are enriched in proteolytic enzymes and can locally degrade the basement membrane, creating a path for invasion. Once the endothelial junctions and the basement membrane are sufficiently compromised, tumor cells can successfully enter the lymphatic vessel lumen. This entire process is facilitated by molecules like VEGF-C, which stimulates lymphatic sprouting and dilation, creating more potential entry points for the tumor cells, and MMPs, which play a crucial role in degrading both junctional proteins and basement membrane components [10].

2.2.2 Extravasation

Extravasation, the crucial process of tumor cells exiting the vasculature and infiltrating surrounding tissues, is a critical step in the metastatic cascade, including perineural invasion (PNI), and tumor self-seeding. As seen in figure 3, this multi-step process begins with circulating tumor cells (CTCs) adhering to the endothelium of blood or lymphatic vessels, mediated by molecules like integrins and E-selectin. Tumor cells, driven by various signaling pathways such as PSGL-1, CCD44v to help with the initial signaling pathway [13] interact with these endothelial cells, adhering to them before subsequently transmigrating through the vessel wall. This transmigration involves the breakdown of the extracellular matrix (ECM) by enzymes such as MMP1 and MMP9, with Fascin-1 also contributing to this migration. The process is facilitated by a complex interplay of adhesion molecules, proteolytic enzymes, and signaling factors. For instance, tumor cells may exploit pre-existing lymphatic vessels or induce the formation of new ones through lymph angiogenesis, facilitated by factors such as VEGF-C, which also promotes vessel sprouting for lymphatic vessel entry. The dilation of lymphatic vessels, driven by tumor-secreted factors, can also contribute to tumor cell entry. Tumor cells can undergo epithelial-mesenchymal transition (EMT), often induced by TGF-β, which allows them to migrate more efficiently and express CCR7, which guides them toward CCL21 produced by lymphatic endothelial cells. Additionally, chemokines such as IL-6 and IL-8 act as attractants for CTCs. Although the specific molecules and pathways involved in PNI extravasation may differ, the fundamental principles of tumor cell-endothelial cell interactions, adhesion, and transmigration likely remain consistent. Once through the vessel wall, tumor cells in-

ISSN 2959-409X

teract with the tumor microenvironment (TME), where factors like cancer-associated fibroblasts (CAFs), hypoxia, and EMMPRIN influence their invasive ability. Finally, these cells may proliferate at a distant site or return to the original tumor site, a process termed self-seeding, involving the chemokine CXCL1, to enhance tumor growth.

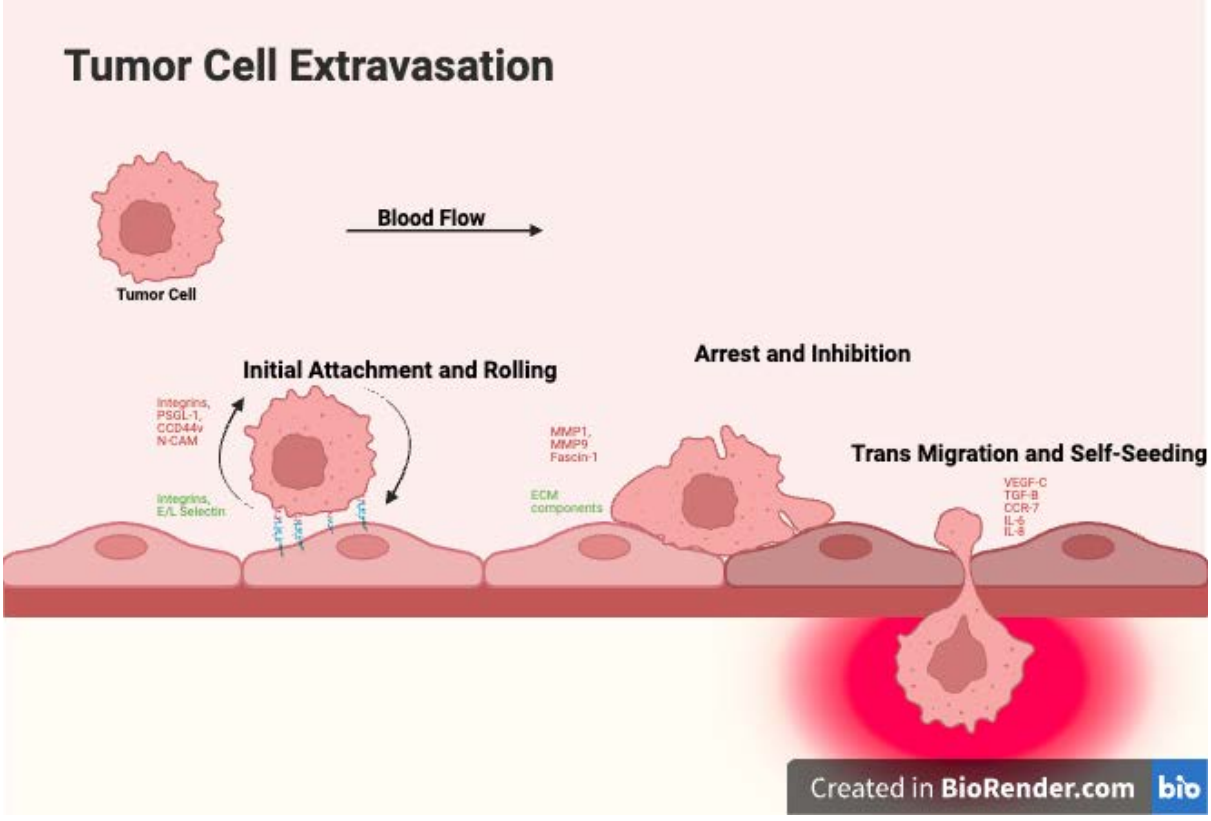

Figure 3. Process of Tumor Cell Extravasation of a Tumor Cell in Mobile Phase

2.2.3 Colonization

Once tumor cells extravasate, they must establish a foothold within the perineural space to grow and spread. This colonization involves interacting with the perineurium, endoneurium, and surrounding extracellular matrix using adhesion molecules such as N-CAM. Tumor cells proliferate, potentially aided by neurotrophic factors released by nerves. Invasion into deeper nerve structures, facilitated by MMPs that degrade the perineurium and endoneurium, enables further migration along nerve pathways. This directional movement, guided by the nerve's architecture and the presence of growth factors, contributes to the spread of tumor cells, potentially leading to distant metastasis. The success of colonization depends on factors like nerve calibre, providing space and resources for growth; tumor cell aggressiveness, influencing invasive and migratory potential; and the state of the immune system, as immunosuppression can hinder the elimination of colonizing tumor cells. Therefore, colonization of the perineural space is a critical step in PNI progression, increasing the risk of local recurrence and distant metastasis.

3. Current Therapies1

To counter these effects, many new therapies and chemotherapeutic drugs have been put to combat against the development of Cancer. One such therapies is the use of Cisplatin, where Cisplatin undergoes aquation to form and create a platinum complex, thereby binding to the DNA, this ultimately prevents the necessary unwinding of DNA at the N7 position of the guanine residues, disrupting the critical functions of the cell cycle. This is seen as DNA lesions by the cellular repair machinery and activate the DNA damage responses (DDR) This response leads to cell cycle arrest and, if the damage is irreparable, can trigger programmed cell death or apoptosis through pathways involving p53 and other apoptotic factors. However, Chemoresistance allows for the upregulation of drug efflux pumps such as P-glycoprotein, increased activation of DNA repair pathways, and alterations in apoptotic signaling. Cancer may also augment the expression of ATP binding cassettes (ABC) transporters, which actively transports cisplatin out of the cell.

4. p53 and Chemoresistance

Chemoresistance is a phenomenon where cancer cells become more unresponsive to chemotherapeutic drugs, this can wither occur from the start of treatment or can be acquired during therapy. This can occur from various drug responses by the cell and the natural selection cells who are more fitted to survive in the environment [14]. Moreover, factors in the Tumor Microenvironment (TME) such as hypoxia or the Extracellular matrix can promote resistance [14]. Thereby introducing chemoresistance among the interplay and microenvironment of cancer cells.

The tumor suppressor gene p53 plays a crucial role in regulating cellular responses to stress, particularly the DNA damage induced by chemotherapy. Wild-type p53 (wtp53) promotes chemosensitivity by inducing cell cycle arrest, DNA repair, or apoptosis in response to drug-induced damage [14]. This is vital for maintaining genomic integrity and preventing the propagation of damaged cells. However, mutations in p53, frequently observed in cancers, can disrupt these functions and contribute to chemoresistance [15,16,17,18]. Mutant p53 (mutp53) can also exhibit a "gain-of-function" (GOF), leading to enhanced tumor cell survival and resistance to treatment [15,16,17].

One of the primary ways p53 influences chemoresistance is through its impact on apoptosis. wtp53 triggers apoptosis, a programmed cell death pathway, in response to irreparable DNA damage [15, 19]. This eliminates damaged cells, preventing tumor progression. However, tumors with p53 mutations or loss of function often fail to undergo apoptosis, which allows the survival and proliferation of drug-damaged cells [16,19]. The apoptotic activity of p53 is mediated by various downstream effectors, such as Bax, a pro-apoptotic protein that permeabilizes the mitochondrial membrane, and caspases, a family of proteases that execute the apoptotic program [15,19].

Another key mechanism of p53-mediated chemoresistance involves the regulation of cell cycle checkpoints. wtp53 can arrest the cell cycle at various stages, providing time for DNA repair before proceeding with replication [6,15,16]. This prevents the accumulation of DNA damage and genomic instability. However, mutations in p53 disrupt these checkpoints, leading to the propagation of damaged DNA and increased genomic instability, ultimately contributing to chemoresistance. The cyclin-dependent kinase inhibitor p21Cip1 is a crucial mediator of p53-dependent cell cycle arrest, inhibiting the activity of cyclin-dependent kinases (CDKs), which are essential for cell cycle progression [6,15,16,20].

Beyond its intracellular functions, p53 also influences chemoresistance through its modulation of the tumor microenvironment (TME) [15,16]. wtp53 can promote a tumor-suppressive TME, while mutp53 fosters a pro-tumorigenic environment that contributes to chemoresistance [14]. For example, mutp53 can enhance angiogenesis through increased secretion of pro-angiogenic factors like VEGF, providing tumor cells with nutrients and oxygen and promoting their survival even in the presence of chemotherapy. Additionally, mutp53 can drive ECM remodeling, which creates a stiffer matrix that protects cancer cells from drug penetration and immune cell infiltration [14]. This is observed in pancreatic cancer, where mutp53-expressing cancer-associated fibroblasts (CAFs) deposit a stiffer ECM, conferring resistance to gemcitabine, a chemotherapy drug.

mutp53 can also contribute to chemoresistance by promoting the maintenance of cancer stem cells (CSCs) [10,15,18]. CSCs, a subpopulation of tumor cells with enhanced self-renewal and drug resistance, are often implicated in chemoresistance and disease relapse. In colorectal cancer, mutp53 has been shown to increase the expression of stemness markers, such as CD44, contributing to enhanced stemness and resistance to 5-FU [6,15,16,20]. This can be further enhanced by the maintenance of a chemoresistant tumor microenvironment

Furthermore, mutp53 can upregulate drug efflux pumps, such as MDR1 and MRP1, which remove chemotherapeutic agents from cancer cells, reducing their efficacy [6,15,16]. This mechanism is observed in ovarian cancer stem cells (OCSCs), where S100B, a protein that inhibits p53, promotes chemoresistance by increasing MDR1 and MRP1 expression [16, 20].

One possible solution to overcome this is the use of p53 reactivation and induction of massive apoptosis through the regulation of Akt activation. Over time, Akt, a serine, thionine kinase is activated by growth factors in a 3-OH-kinase (PI3K)-dependent manner. This overactivation in chemoresistant cancer cells sensitizes wt-p53 through Cisplatin (CDDP)-induced apoptosis.

On its own, CDDP is unable to treat cancer cells effectively due to its ability dependent on the cell's ability to undergo apoptosis because the cell develops resistance to apoptosis due to p53 gene mutations. This activates Akt, serine/threonine kinase, is implicated in cell proliferation and survival. This overexpresses the PI3K-Akt Pathway which releases MDM2 into the nucleus, suppressing p53 and inhibiting its function and promoting resistance. Therefore, the cell gains resistance to this mechanism and therefore CDDP can't induce apoptosis.

PRIMA-1 is a low molecular weight compound which reactivates and restores the function of p53 though the covalently bonding to the core domain and induce apoptosis in tumor cells. This restores its wild-type conformation. As seen in figure 4, PRIMA-1 directly induces apoptosis

ISSN 2959-409X

into tumor cells, increasing the effectiveness of p53 since mutant p53 often leads to resistance to apoptosis. On its own, CDDP is unable to treat cancer cells effectively due to its ability dependent on the cell's ability to undergo apoptosis. With this, studies show that p53 is more effective at inducing apoptosis in mutant p53 cells than that of wild type p53.

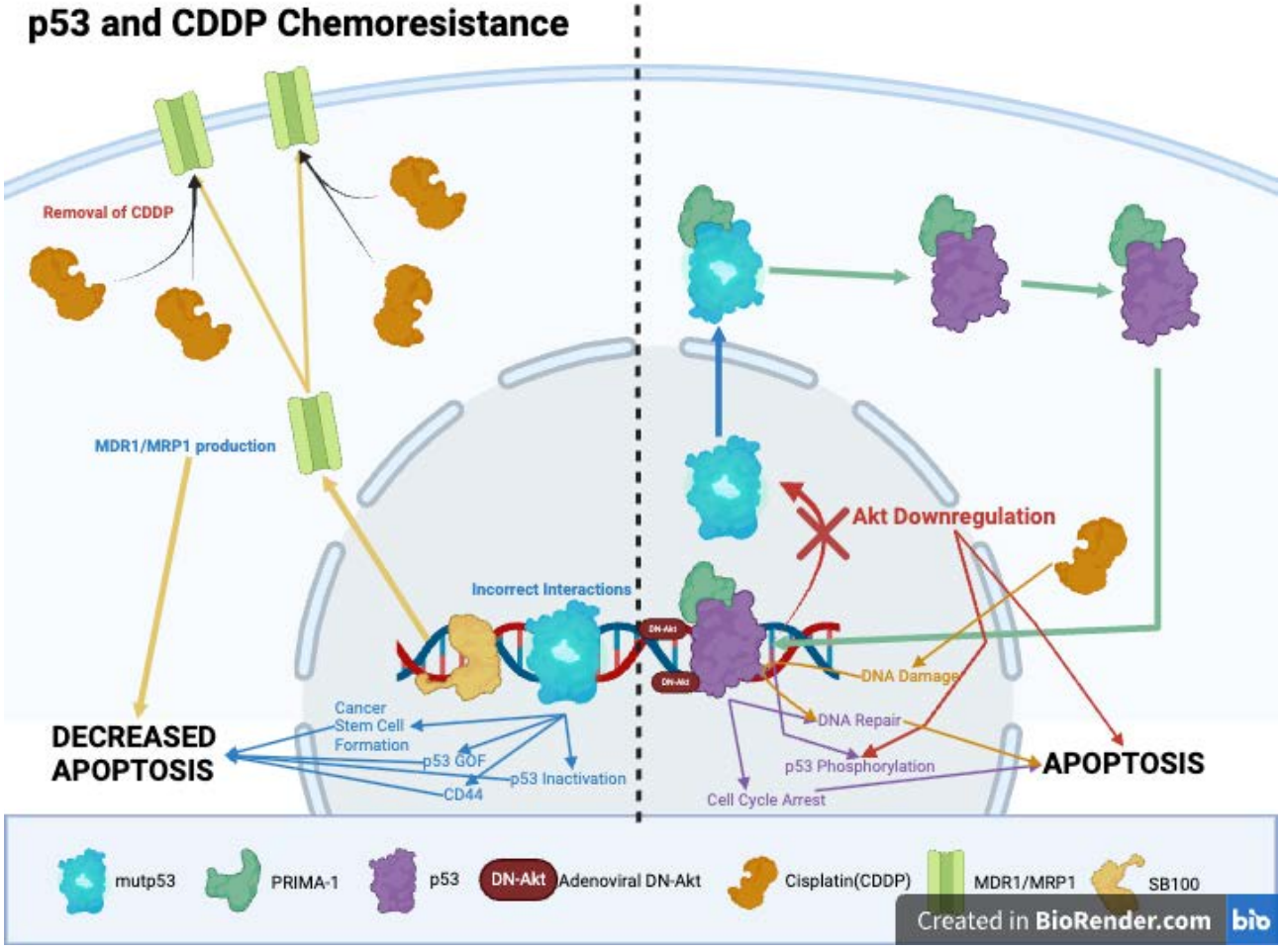

Figure 4. Mechanism of Action of CDDP and p53 on the Apoptosis of a Tumor cell.

Surprisingly, this mechanism works more effectively with the function of Dominant-Negative Akt (DN-Akt) regulating the function of Akt. This is because it can effectively increase p53 Phosphorylation at Serine15(Ser15) [21]. According to the study conducted by [16], the Apoptosis rate was evaluated with difference concentration through the setup of of PRIMA-1 (0–10 μM) plus CDDP (0 or 10 μM) using adenoviral DN-Akt or LacZ (as control). They then set up PRIMA-1 and CDDP when Akt is down-regulated (DN-Akt) against PRIMA-1 alone and Akt down-regulated (DN-Akt) as well as LacZ as a control Group with normal Akt function.

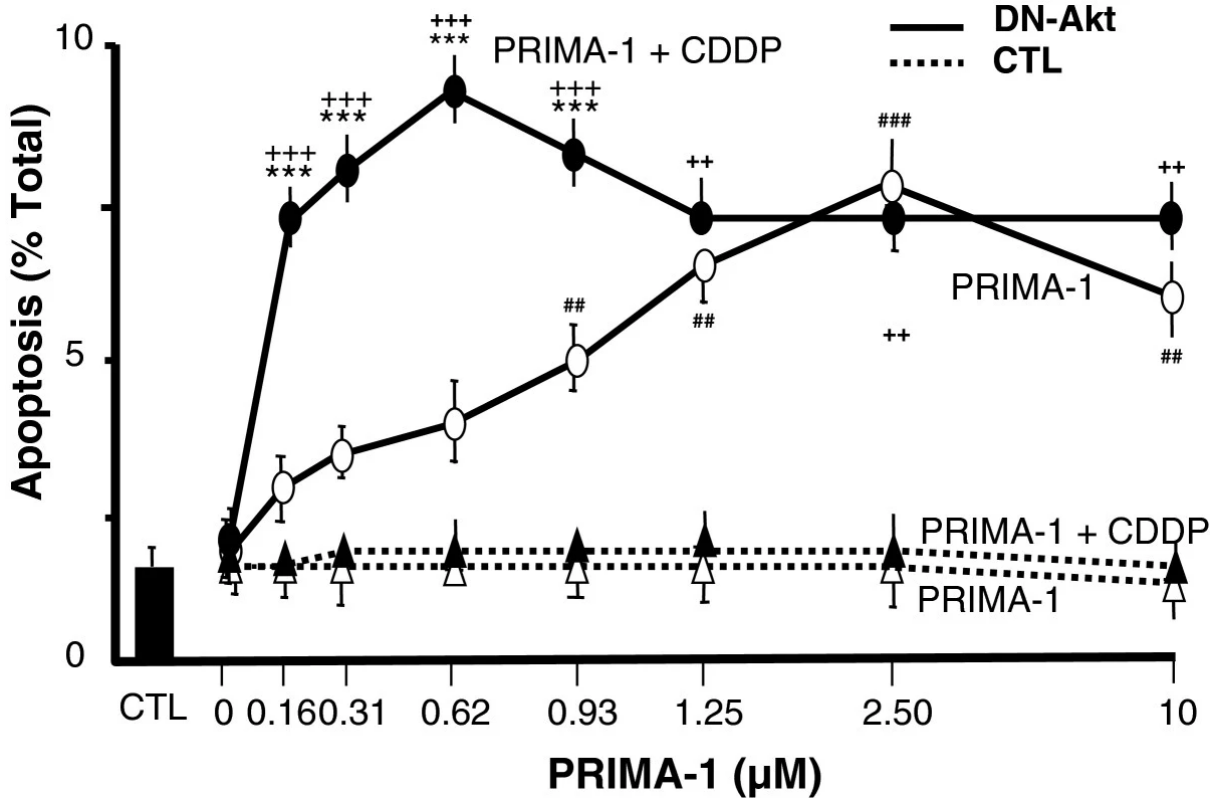

Figure 5. The effect of different concentration of PRIMA-1 on Akt down Regulation (Apoptosis rate was evaluated with difference concentration of PRIMA-1 (0–10 μM) plus CDDP (0 or 10 μM) using adenoviral DN-Akt or LacZ (control). *** P<0.001; PRIMA-1+CDDP & DN-Akt vs. PRIMA-1 & DN-Akt; +++ P<0.001, ++ P<0.01; PRIMA-1+CDDP & DN-Akt vs. PRIMA-1+CDDP & CTL; ### P<0.001, ## P<0.01; PRIMA-1+CDDP & DN-Akt vs. PRIMA-1 & CTL. Results are expressed as mean ± SEM of three independent experiments.) (Adapted from [21])

As seen in figure 5, the control group exhibited minimal apoptosis across all PRIMA-1 concentrations. While PRIMA-1 alone showed a modest increase in apoptosis at higher concentrations, the most significant increase occurred in the presence of DN-Akt, demonstrating a clear dose-dependent relationship and suggesting a synergistic effect. Conversely, combining PRIMA-1 with CDDP in the control group, did not significantly enhance apoptosis beyond the levels observed with CDDP alone, indicating that DN-Akt greatly enhances the Apoptosis rate and effectiveness.

5. Conclusion

In conclusion, this paper mostly talks about regulating and preventing Cancer through the apoptosis of cancer cells. Through the enhancing of p53 and its functions, common obstacles needed for p53 regulation can be negated and prevented. Therefore, allowing the p53 gene to continue doing its job. In the future, it would be better if more pathways and methods of preventing cancer overgrowth without using p53, since p53 by itself can be very vulnerable.

6. Discussion

The study of cancer progression and therapeutic resistance remains a multifaceted challenge in oncology. Our understanding moving from early conceptualizations of tumor spread to detailed molecular and microenvironmental insights. Initially, theories of metastasis, like Paget's "seed versus soil" hypothesis and Ewing's anatomical model, provided foundational perspectives [3]. These were later refined by models such as Bross's metastatic cascade, which posited non-random, stepwise dissemination [3]. Current research continues to unravel the mechanisms of cancer spread, with recent findings challenging the classical notion that metastasis is solely a late-stage event. Indeed, the capacity to metastasize appears to be an intrinsic property of specific malignant cell subpopulations, highlighting metastatic heterogeneity within the primary tumor.

ISSN 2959-409X

References

1. Han, L., Guo, X., Du, R., Guo, K., Pei, Q., & Bian, H. (2022). Identification of key genes and pathways related to cancer-associated fibroblasts in chemoresistance of ovarian cancer cells based on geo and tcga databases. Journal of Ovarian Research, 15(1). https://doi.org/10.1186/s13048-022-01003-2

2. Malhotra, J., Malvezzi, M., Negri, E., La Vecchia, C. and Boffetta, P. (2016). Risk factors for lung cancer worldwide. *European Respiratory Journal*, 48(3), pp.889–902. doi: https://doi.org/10.1183/13993003.00359-2016.

3. Chang, P. C., Fischbein, N. J., McCalmont, T. H., Kashani-Sabet, M., Zettersten, E., Liu, A., … & Weissman, J. L. (2004). Perineural spread of malignant melanoma of the head and neck: clinical and imaging features. American Journal of Ophthalmology, 137(6), 1173-1174. https://doi.org/10.1016/j.ajo.2004.04.040

4. Kim, M. Y., Oskarsson, T., Acharyya, S., Nguyen, D. X., Zhang, X. H., Norton, L., … & Massagué, J. (2009). Tumor self-seeding by circulating cancer cells. Cell, 139(7), 1315-1326. https://doi.org/10.1016/j.cell.2009.11.025

5. Bi, Y., Chen, Q., Yang, M., Xing, L., & Jiang, H. (2024). Nanoparticles targeting mutant p53 overcome chemoresistance and tumor recurrence in non-small cell lung cancer. Nature Communications, 15(1). https://doi.org/10.1038/s41467-024-47080-3

6. Zhou, Y., Nakajima, R., Shirasawa, M., Fikriyanti, M., Zhao, L., Iwanaga, R., … & Ohtani, K. (2023). Expanding roles of the e2f-rb-p53 pathway in tumor suppression. Biology, 12(12), 1511. https://doi.org/10.3390/biology12121511

7. Zhou, Y., Nakajima, R., Shirasawa, M., Fikriyanti, M., Zhao, L., Iwanaga, R., … & Ohtani, K. (2023). Expanding roles of the e2f-rb-p53 pathway in tumor suppression. Biology, 12(12), 1511. https://doi.org/10.3390/biology12121511

8. Schmitt, C. A., Fridman, J. S., Yang, M., Baranov, E., Hoffman, R. M., & Lowe, S. W. (2002). Dissecting p53 tumor suppressor functions in vivo. Cancer Cell, 1(3), 289-298. https://doi.org/10.1016/s1535-6108(02)00047-8

9. Pang, M., Georgoudaki, A., Lambut, L., Johansson, J., Tabor, V., Hagikura, K., … & Fuxe, J. (2015). Tgf-β1-induced emt promotes targeted migration of breast cancer cells through the lymphatic system by the activation of ccr7/ccl21-mediated chemotaxis. Oncogene, 35(6), 748-760. https://doi.org/10.1038/onc.2015.133

10. Kim, M. Y., Oskarsson, T., Acharyya, S., Nguyen, D. X., Zhang, X. H., Norton, L., … & Massagué, J. (2009). Tumor self-seeding by circulating cancer cells. Cell, 139(7), 1315-1326. https://doi.org/10.1016/j.cell.2009.11.025

11. Wyk, H. C. v., Going, J. J., Horgan, P. G., & McMillan, D. C. (2017). The role of perineural invasion in predicting survival in patients with primary operable colorectal cancer: a systematic review. Critical Reviews in Oncology/Hematology, 112, 11-20.

12. De Pergola, G. and Silvestris, F. (2013). Obesity as a Major Risk Factor for Cancer. Journal of Obesity, 2013, pp.1–11. doi: https://doi.org/10.1155/2013/291546.

13. Unger, T., Sionov, R., Moallem, E. *et al.* Mutations in serines 15 and 20 of human p53 impair its apoptotic activity. *Oncogene* 18, 3205–3212 (1999). https://doi.org/10.1038/sj.onc.1202656

14. Souza, L. C. d. M. e., Faletti, A., Veríssimo, C. P., Stelling, M. P., & Borges, H. L. (2022). P53 signaling on microenvironment and its contribution to tissue chemoresistance. Membranes, 12(2), 202. https://doi.org/10.3390/membranes12020202

15. Malhotra, J., Malvezzi, M., Negri, E., La Vecchia, C. and Boffetta, P. (2016). Risk factors for lung cancer worldwide. *European Respiratory Journal*, 48(3), pp.889–902. doi: https://doi.org/10.1183/13993003.00359-2016.

16. Fang, D., Hu, H., Zhao, K., Xu, A., Yu, C., Zhu, Y., … & Wu, X. (2023). Mlf2 negatively regulates p53 and promotes colorectal carcinogenesis. Advanced Science, 10(26). https://doi.org/10.1002/advs.202303336

17. Gao, X., Zheng, X., Zhang, Y., Dong, L., Sun, L., Zhao, N., … & Wang, Y. (2022). Deficient or r273h and r248w mutations of p53 promote chemoresistance to 5-fu via tcf21/cd44 axis-mediated enhanced stemness in colorectal carcinoma. Frontiers in Cell and Developmental Biology, 9. https://doi.org/10.3389/fcell.2021.788331

18. Yang, T., Cheng, J., You, J., Yan, B., Liu, H., & Li, F. (2018). S100b promotes chemoresistance in ovarian cancer stem cells by regulating p53. Oncology Reports. https://doi.org/10.3892/or.2018.6527

19. He, Y., Rajantie, I., Pajusola, K., Jeltsch, M., Holopainen, T., Ylä-Herttuala, S., … & Alitalo, K. (2005). Vascular endothelial cell growth factor receptor 3–mediated activation of lymphatic endothelium is crucial for tumor cell entry and spread via lymphatic vessels. Cancer Research, 65(11), 4739-4746. https://doi.org/10.1158/0008-5472.can-04-4576

20. Unger, T., Sionov, R., Moallem, E. *et al.* Mutations in serines 15 and 20 of human p53 impair its apoptotic activity. *Oncogene* 18, 3205–3212 (1999). https://doi.org/10.1038/sj.onc.1202656

21. Kobayashi, N., Abedini, M. R., Sakuragi, N., & Tsang, B. K. (2013). Prima-1 increases cisplatin sensitivity in chemoresistant ovarian cancer cells with p53 mutation: a requirement for akt down-regulation. Journal of Ovarian Research, 6(1). https://doi.org/10.1186/1757-2215-6-7