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Hypoxic-Sensitive Nano-Carriers for Anti-Neoplastic Drug Delivery: New Perspectives

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Abstract
Since the inception of Anti-neoplastic drug delivery in the 1950s oncologists and researchers have revolutionized the field of diagnostics and treatment with novel therapeutic modalities. Tumor hypoxia remains the first and foremost factor contributing to a resistance in anti-neoplastic drugs, ionized radiation and chemotherapy by solid tumors. The laps in vasculature in solid tumors promulgates tumor resistance by inhibiting the hypoxia inducible factor (HIF) within the tumor. It is on this basis that the selectivity of hypoxic cells are datelined. Based on clinical studies it was revealed that hypoxia does not occur in healthy human tissue; thus, paving the way for the exploitation of hypoxia as an advantage in creating novel therapeutic methods of detection and treatment for solid tumors with an added advantage of nano-science. The biological application of nano-carriers is a fast developing area of nano-science that is expected to realize new and innovative possibilities in the diagnosis and treatment of human cancers. Cancer diagnostics using nano-carriers entails the use of fluorescent nanoparticles for a multiplex of simultaneous profiling of tumor bio-markers. These fluorescent probes are also used for detection of multiple RNA matrices and genes within in-situ hybridization. It is expected that in the very near future, the application of conjugated nanoparticles in anti-neoplastic tumor detection and drug delivery will enable oncologists and scientists to pinpoint and identify cancer-related proteins on tumor surfaces, providing a new method of analysis; thus, creating personalized treatment methods for individual tumors. Nano-carriers possess an extraordinary possibility as contrast agents for cancer cell detection in vivo, and for monitoring the response to treatment of select cancers.
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Introduction

Modern day anti-neoplastic therapy, tumor identification and drug delivery in particular has seen a revolution in new methods and perspectives when compared to traditional methodologies. A significant part of this phenomenal change is the increase of the therapeutic index of chemo-therapeutics and prodrugs. Although neo-blastopic cells are significantly susceptible than normal cells, the effects of chemo-therapeutics and prodrugs cause significant damage to normal cells and tissue because of the non-selectivity/non-specificity of the drugs. Hypoxia and toxicity present themselves as pivotal constraints in the use of this modality thus propagate a reduction in the mortality of cancer patients .The presence of regions of hypoxia is one of the largest constraint in drug delivery because it enables necrosis due to a laps in tumor vasculature. A more modernized attempt surrounds a more selective approach for killing cancer cells with the use of more specific targeting which will result in the sparing of normal uninfected cells .To achieve these targets, the focus is placed on developing novel nano-carriers for drugs that are presently on the market as well as upcoming drugs as a means of defining specific targets based on molecular as well as genetic changes in neoblastopic cell vasculature and its associated stromas.

Hypoxia in tumor cells results from (inefficiency of blood supply in tumor micro environment) and places a limit on the effectiveness of anti-tumor drugs and other treatment modalities. The use of prodrugs combined with nano-medicine that stimulates active species in hypoxic tissue and hypoxic tumor environment via selective biological reduction poses as a viable method of forcing cancerous tumors into remission. Over the years, based on different combinations in pharmaceutical chemistry a great deal has been garnered about the various constructs of prodrugs in nano-medicine that makes them suitable for (efficient extracellular diffusion ,suitable reduction in tumor micro environmental kenetics and effective biological profiling of active specie) their effective application to oncology. A key characteristic of prodrugs in nano-medicine amplifies diffusion across hypoxic tumor micro environments. This has assisted the development of novel treatment modalities that result in the obliteration of cancer cells and promise a new added novelty to the evolution of anti-neoplastic drug creation. In hypoxic cells, the cell cycle division of these cells are not dependent on oxygen for proliferation; the application of nano-medicinal approaches will see the diffusion of hypoxic-efficient areas in solid tumors resulting in an out of cycle hypoxic cell death via the Warbury Effect. Hence, the elimination of the hypoxic

cells will occur when they proliferate.

This review addresses the biological application of modified nano-carriers in anti-neoplastic research. It includes the recent development of molecular approaches being used in the detection and targeting of hypoxic tumors. The methods includes: the use of modified nano-carriers to identify tumors as well as to deliver anti-cancer drugs to solid hypoxic tumors as a means of inducing cell death via hypoxia.

1.Tumor Vasculature ,Hypoxia and Necrosis

Tumor vasculature

Neoblastopic cell mass derive its nutrients via passive diffusion until it reaches a suitable size. As it burgeons, the formation of new blood vessels (angiogenesis) is necessary to deliver a supply of nutrients for the fast expanding tumor mass. Tumor angiogenesis initiates a chain of biological signaling. The process is disorderly and causes poor vascularization resulting in areas of necrosis; hence, hypoxia and poor drug distribution occur in these areas. Other areas in the tumor are properly vascularized and susceptible to anti-cancer prodrugs. The vascular network of vessels in a tumor are abnormal and branches abhorrently into binds and loops proving it to be very tortuous. The presence of abnormalities in the basement membrane in the tumor poses several challenges such as a decreased number of pericytes which is needed for the lining rapid proliferating endothelia cells in the fast expanding mass. These abnormalities result in leakages in the endothelium of the tumor and result in enhanced permeability for the diffusion of molecules across the vessel walls into the intistitium surrounding tumor cells. The leaky endothelial gaps range between 100-780 nanometers depending on the nature and type of tumor, As opposed to tightly packed endothelial cells in normal vessels which are typically between the ranges of 5-10 nanometers in size.

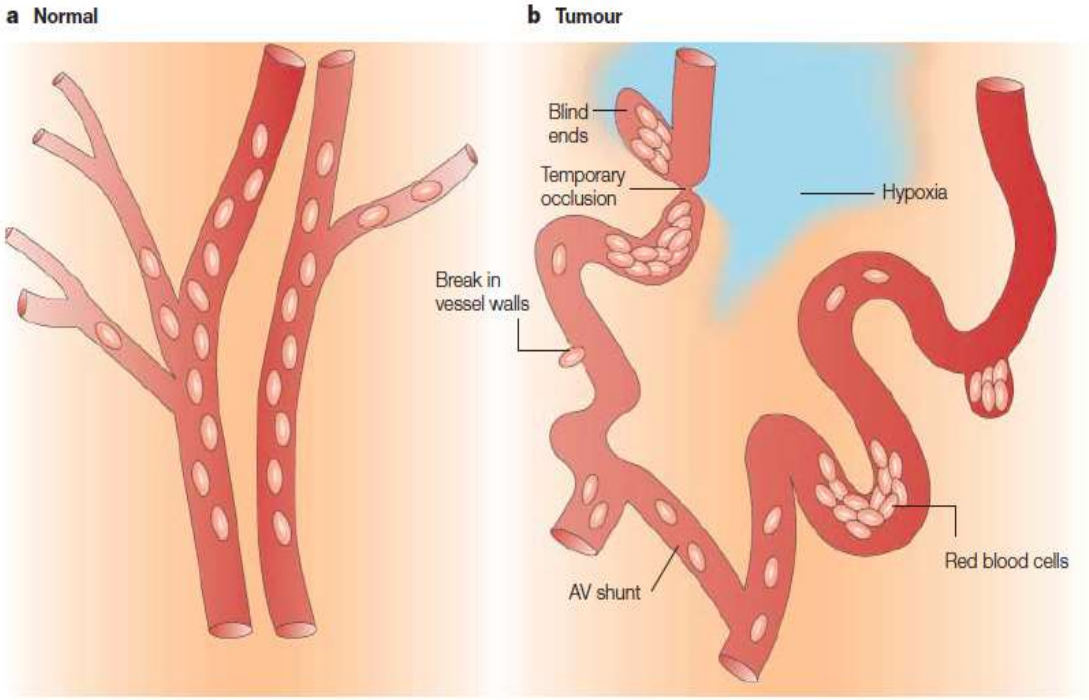

Figure 1 : Shows a vasculature network of Normal Tissue and Hypoxic Tumor tissue. Normal Tumor vasculatures clearly indicate the supply of oxygen as is vital for nutrient as well as gas transport. (A) Depicts an active normal/vasculature with vessels close to the surface to ensure adequate nutrient and oxygen supply to the cells . Hypoxic Tumor vasculature (B) depicting areas of hypoxia and necrosis , the vessels are dilated tortuous and chaotic and as seen are often far apart from each other as well as far from the tumor surface . Oxygen deficient areas are consequently characterized by necrosis because these areas are deficient in blood vessels. In addition to necrosis the area is diffusion limited thus resulting in the formation of hypoxic tumors.

1.1Tumor Hypoxia and Necrosis

Hypoxic regions in tumors are characterized by low oxygen content due to a lack of viable blood vessel and necrosis (Figure 1) .As opposed to normal tissue solid hypoxic tumors lack the necessary vasculature rendering them highly heterogeneous thus exhibiting low oxygen tension.[2] In hypoxic conditions neoplastic tumor cells are at a higher resistance towards therapies and is directly associated with its proliferation.

When compared to normal cells, cells under hypoxic conditions display an elevation of reductive enzymes which intern result in reductive stress.[2] A change in vasculature creates changes in the Ph as well as the

glucose concentration of the tumor . These alterations in the micro environment cause the onset of a variety of changes such as metabolic, biological as well as gene expression changes in the tumor.
Through research its known that mammalian cells possess the ability to create a HIF (Hypoxic deducible Factor).[3]

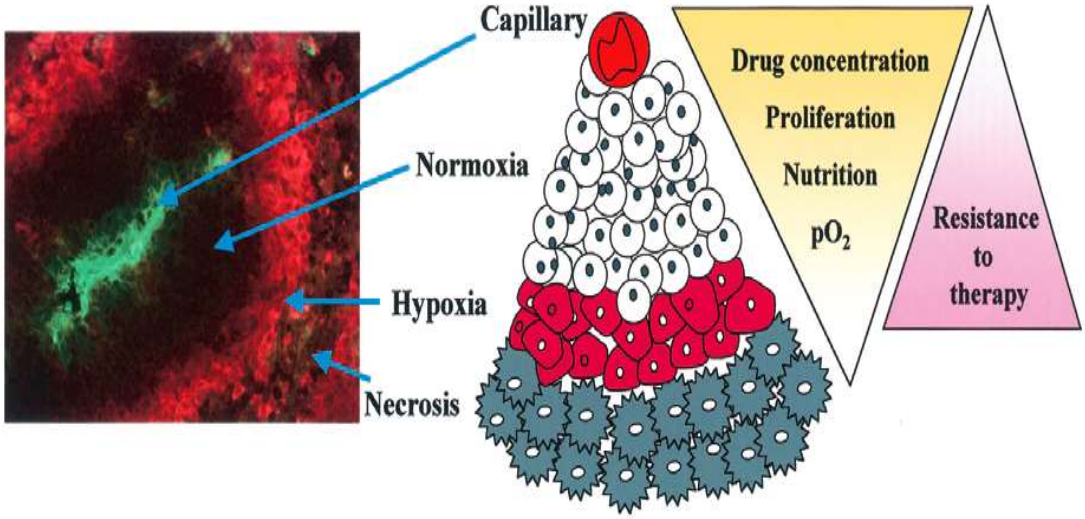

Figure 2 . Hypoxia in solid Human tumors. The pictograph to the left shows a cross-section of an experimental human colon tumor (HCT116) treated with an antibody CD31 (green), the tumor was also treated with an endothelial cell marker, and pimonidazole (red), and a hypoxic marker. Visualization was made possible by adding secondary antibodies conjugated to fluorescent proteins which intern resulted in the corresponding color. The animation in the middle depicts tumor cord surrounding a capillary. With an increase in distance from the capillary/blood supply, cellular proliferation, anticancer drug concentrations; oxygen as well as nutrition decrease as well, while resistance to ionized radiotherapy and chemotherapy increases considerably.

1.2 Oxygen concentration in Solid Tumors and normal Tissue

Oxygen Heterogeneity is a typical characteristic of solid tumors; however, this characteristic differs among patients and individual tumors. The oxygen stability is measured with the aid of biochemical techniques such as polarographic oxygen electrodes that depend solely on the mechanisms of antibody detection of nitromidazole based adducts present in hypoxic tissue. With the use of oxygen electrodes it was demonstrated that solid human tumors possess regions where the oxygen concentration is significantly lower thus resulting in lowered oxygen tension.[11] Vast differences are observed in normal normoxic tissues whereas tissue oxygenation in primary human tumors are not promulgated only by metabolic demands. However, the po2 of the tumor tissue is predominantly low and heterogeneous. Hypoxia is also directly associated with the tumor size. A tumor which possesses a volume of ccm3 and above typically has a pronounced region of hypoxia .Oxygen tension measurements in solid human tumors often show median oxygen tension ranges from a low of 1.13-3.9%(10-3-mmHg) to a high of 3.1-8.7%(24-66mmHg).The concentration detected in normal tissue is exceptionally higher, it ranges from a low of 3.1-8.7% O2 (24-66mmHg) [14 this gives researchers an added advantage of creating novel modalities as a means of detecting and treating tumors.

1.3 Biological Applicant of Nano-carriers in Anti-neoplastic Drug delivery

The biological application of nano-carriers in anti-neoplastic drug delivery presents itself as novel therapeutic technique that increases the possibilities of diagnostics and treatment of cancers in humans.[1] The structure of nano-Carriers gives it several advantages making it a suitable transport modality in anti-neoplastic research. The unique and unconventional physical and chemical properties of nanoparticles, such as small size, large surface area, low mass ratio, poor water solubility and high reactivity increase the advantages of nanoparticles to overcome some of the limitations of treatment and diagnostic medicine.

Nanotechnology and its application in medicine (Nano-Medicine) has seen several revolutionary developments in the field of oncology. The production and application of nano-materials at various nano scales levels have enabled the transportation of substrates to target molecules invivo.[5] The size of a nanoparticle is directly associated with its physiochemical properties which is a key characteristic when used in biological imagery ,biomass targeting as well as substrate delivery .

Nano-carriers size (nanometer) provides added advantage in creating new methods for anti-neoplastic drug delivery. Nano-carriers are able to penetrate fenestrations within solid tumor vasculature allowing direct cellular access. These particulate dispositions allow superb modification for binding to cancer cell membranes, the

microenvironment, or to cytoplasmic or nuclear receptor sites.[17] This feature results in the delivery of high drug concentrations to the targeted cancer cell, with reduced toxicity to the surrounding normal tissue. [24] At present several drugs of this engineered format are being used in clinical practice, some of these drugs including liposomal doxorubicin and albumin conjugate paclitaxel.

The modified mediated carrier paclitaxel has shown paramount efficacy in taxane resistant cancers, a highly relevant methodology in prostate cancer treatment [25], where taxanes are the treatment modality being used. This technology provides a plethora of compelling novel therapeutic approaches for targeting and delivery of high concentration drug to hypoxic cancer cells with reduced injury of normal cells and the micro-environment.

2.0 Nano-carriers for in vivo Anti-neoplastic drug delivery

The investigation of polymeric nano-carriers for cancer therapy has been extensive through the years[55] the unique properties and structures nano-carriers present an chance at maximizing solubility , bioactivity and bioavailability of anti-neoplastic drugs for select and personalized cancer therapies .[61] [66] [71] With these properties in mind, polymeric nano-carriers are advantageous for systematic administration . With invivo administration of these nano-carriers , particles smaller than 5 nm are rapidly removed from the body by renal excretion; however, micro particles are easily filtered by the sinuses and cleared by the reticuloendothelial system of the liver and spleen respectively . From the lower Gastrointestinal tract nano-carriers that are 10-500 nm can circulate in the blood stream for prolonged periods adding contentious delivery of anticancer drug to tumor tissue through its EPR effect based on its impaired lymphatic system and leaky vasculature. Maximization of prodrug efficacy can be achieved based on several simultaneous stimuli-responsive linker of moieties which have been incorporated into the outside channel of amphiphilic copolymers thus forming a a new self-assembled nano-particle adding contentious drug concentration in the tumor micro environment.

Thambi et al .Recently developed hypoxic responsive nano-carriers (HR-NPs) for drug transport in cancer therapies. The nano-carriers were synthesized by conjugating 2-nitromidzole derivatives to a carboxyethyle dextran backbone. Because of the amphiphilic nature the conjugates, self-assembly into micelles in an aqueous solution was possible using a 2,6 conjugation modality .Anticancer drug Doxorubicine (DOX) which is an anthracycline was successfully incorporated into the nano-carriers .Stability of the HR-NPs was achieved under physiological condition and the drug was released sustainably. The release of the drug was enhanced notably under hypoxic condition. Under microscopic observation it was observed that the HR-NPs has the ability to effectively deliver DOX into the SCC7 cells under hypoxic conditions bringing about high concentrations of toxicity. In vivo bio distribution, microscopy also exhibited marked indications that the HR-NPs selectively accumulated at the hypoxic tumor micro environment and the tumor tissue by extension. By the presence of accumulation it is safe to infer that heavy anti-cancer activity was observed as well.

According to another study conducted by Park et al . Hydrophobic nitrobenzyl derivatives were used as hypoxia sensitive nano-carriers for cancer drug delivery. The nano-carrier moiety was conjugated to block copolymers which is based on polyethlyn glycol (PEG) and polylysine. Cognizant of the amphilhilic disposition of the block copolymer, it was incorporated effectively into the micelle DOX under aqueous conditions. The micelles loaded with DOX exhibit rapid inter cellular release of the DOX under hypoxic conditions [55]. The opposite was seen in normoxic conditions where the drug encapsulated nano-carriers were rendered inactive. This further implies that the nitrobenzyl derived hypoxic sensitive nano-carriers impregnated with anticancer drugs possessed a greater potential for use in hypoxic conditions. The inference can also be drawn that they possess a greater potential when used in select cancer therapies. Subsequently, Ahmed et al also developed polyethylene glycol based nano-carriers for hypoxic sensitive conjugate nitromidazole derivatives. They were conjugated onto a polyethlyn glycol backbone copolymer. [47] DOX was also incorporated into the hydrophilic core of the nano-carriers via an aqueous solution. The results showed that the DOX-loaded nano-carriers triggered their drug release potential while subjected to a hypoxic micro environment .

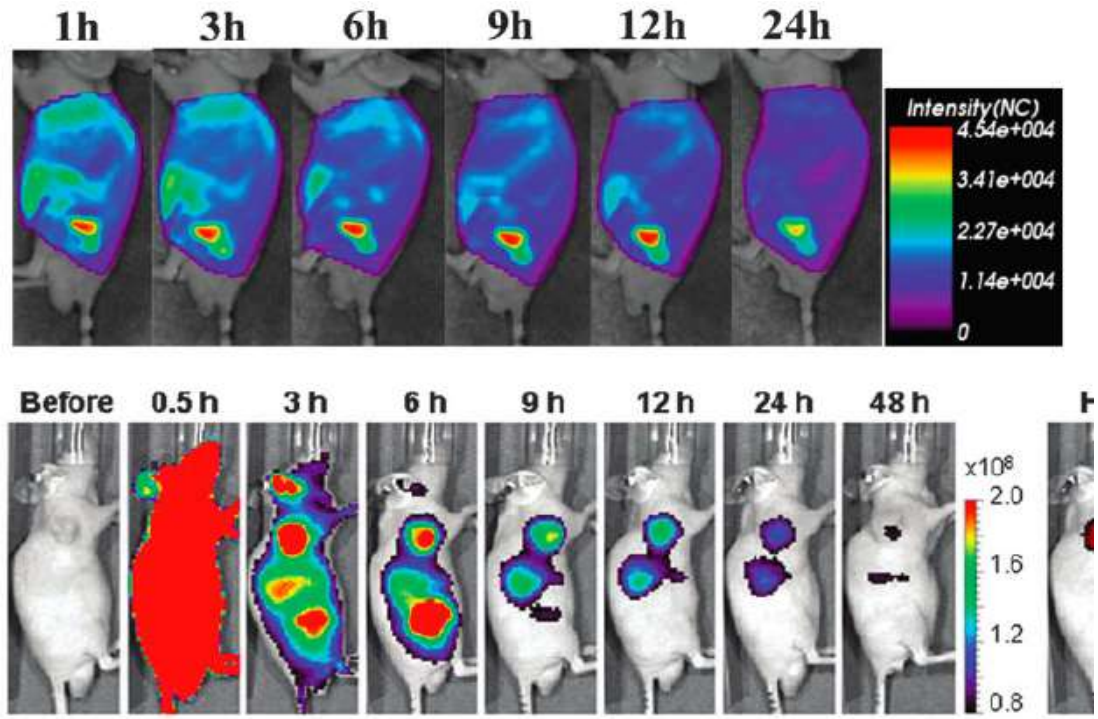

Figure 3: (A) in vivo biodistribution of the pimonidazole-conjugated Cy7-based hypoxia fluorescent probe.(B) In vivo Imagery of SSC7 tumor baring mice inculcated with HR-NPs . Whole body imagery indicates tumor disposition and nano-carriers impregnated with DOX Cy5.5 labelled HR-NPs.

Recent research development around hypoxic-responsive prodrug nanoparticles has indicated their biological activity of drug activation in hypoxic tumor micro environments. Zhu et al synthesized a hypoxic activateable phototrigger-conjugate nano-carriers for rapid selective dispersion of anticancer drugs. The nano-carriers were synthesized by incooperating nitromidzole and cumarin-encapsulated anti-cancer drugs inside glycol chitosan. Dismissal of the photoexcitation effect of the coumarin dye was observed in normoxic tissue as well as no drug dispersal was observed .The photoinduced electron transfer (PET) of coumarin to the electron receptive nitromidazole radicle makes them suitable for the syntheses of the hypoxic-responive nano-carriers. When subjected to hypoxic micro environments of solid tumors, the reaction produces amines as the end product as the nitro group of the nitroimidazole is reduced. This reduction of the nitro group to amine resulted in the activation of the light-induce cleavage of the drug-coumarine linker. According to other studies different hypoxia -triggered prodrugs conjugate were synthesized through the incorporation of azobenzene links between PEG-hexanethiol and combretastatin . [67] The nano-carriers are amphiphilic and were able to self-assemble into micelles in a physiological fluid , they possessed the remarkable ability to specifically release their drug load when subjected to hypoxic tumor micro environments.

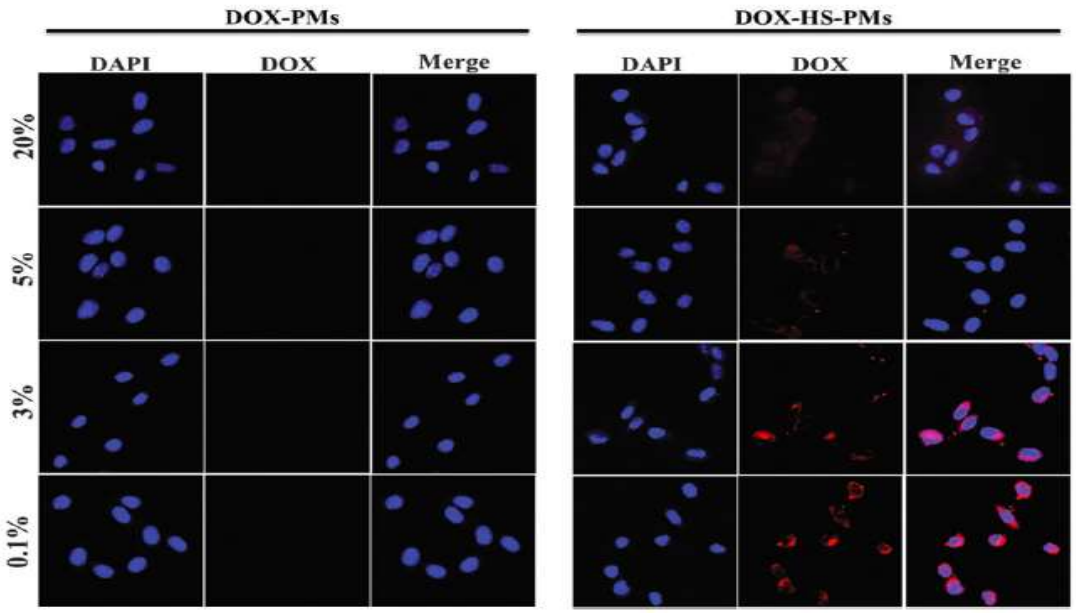

Figure 4 : Micro environment intracellular drug offloading and dispersion . Release disposition of DOX-loaded control micelles (DOX-PMs) and hypoxic sensitive micelles (DOX-HS-PMs) . The DOX loaded nano-carriers were inoculated into a SSC7 cell environment under normoxic and hypoxic conditions and observed over a 12 hour period under different degrees of oxygen exposure.

3.0 Modulating Tumor Hypoxia for Enhance Cancer therapy .

Malignant proliferation of tumor cells when linked to modulation of hypoxia in the enchantment and treatment of cancers, sees the development of new blood vessels/ pathways with poor blood circulation. This is due to the fact that the interior vasculature of solid tumors would have an inefficiency of oxygen which is needed to regulate the increasing metabolic processes which are necessary requirements for cellular proliferation. A disposition in oxygen demand would more or likely result in chronic hypoxia. Hypoxia greatly influences the results of therapies and therapeutic methods which are constructed based on oxygen involvement which is a critical element in the fight against cancer. Therapies such as radiotherapy and photo-dynamic therapy (PDT) are light triggering modalities which require the transfer of energy from light to oxygen within solid tumors. This light to oxygen transfer process triggers cytotoxic singlet oxygen (1O2) with the corresponding photo-synthesizer.

The use of nano-carriers ameliorate hypoxia with the addition of different mechanisms can be deemed as a useful strategy to achieve enhanced oxygen presence in therapeutic cancer treatment methods. Tumor hypoxia can be ameliorated by improved blood flow into the tumor by decompressing the blood vessel with drugs. The use of oxygen rich nano-carriers increases tumor oxygenation status, promulgating in situ oxygen generation through decomposition of endogenous H2O by catalytic nano-carriers.

PFC are known for their high oxygen stability. Chen et al conducted an experiment which saw the diffusion of oxygen across a concentration gradient within solid tumors. The results demonstrated that PFC based nano-carriers can work as an effective oxygen shuttle for tumor oxygenation as they are able to release the payload when exposed oxygen deficient tissue in solid tumors . In this work the PFC was impregnated with hallow Bi2Se3 nanopar-ticles . The particles were saturated with oxygen , PEG-Bi2Se3. These nanoparticles demonstrated a burst of oxygen when exposed to oxygen deficiency tissue, leading greatly to improved tumor oxygenation and enhanced cancer drug absorption efficiency.

Zhuang et al subsequently did work on HSA stabilized nanodroplets (nano-PFC). The nanodroplets were synthesized to possess stable oxygen solubility and hypoxic environment oxygen release. The nano-PFC was injected into tumor bearing mice after which min-hypoxic breathing treatment was conducted at the tumor site at the same time. The process demonstrated that the nano-PFC would absorb oxygen in the lungs then transport this oxygen via blood circulation, and effectively release the oxygen in hypoxic tissue micro-environments. These results could give hope to therapeutic outcome and could see marketable results and enhancements in the fight against solid tumor cancers.

4 Hypoxia and the Warbury Effect

Hypoxia also contributes to alterations in cancer cell where by an increase in glycolyic activity causes metabolic

alterations in solid tumors via the predisposed anaerobic environment in tumor tissues. This concept remains controversial as it is believed to have promulgated the warbury Effect. In physiological constraints the creation of adenosine triphosphate ATP via oxidative phosphorylation within a cells mitochondria can be considered an effective metabolic process whereas products from ATP molecules reduces the amount of glucose needed for cellular proliferation in solid tumor cells . However, solid tumor cells are able to adapt to differential pathways if their ability to generate ATP via mitochondrial oxidative phosphorylation is compromised. One chief pathway is an increase in glycolytic activity to maintain energy supply. In this light mitochondrial respiration functions will be altered negatively as multiple sub-variables such as mutation in mitochondrial DNA , (mtDNA), electron transport chain mutation , and unsanctioned enzyme expression which is needed for energy metabolism . These sub-variables will result in insufficient oxygen disposition within solid tumors thus resulting in a hypoxic cellular micro environment .

It is a well known concept that displacement loops exist within mtDNA strands, and gene coding of the 13 most important proteins for mitochondrial respiration complexes without introns are directly dependent on these displacement loops.

mtDNA mutation is likely to causes alterations of coding proteins which intern disrupts the respiratory chain function . With this concept in mind the frequency of mtDNA observable in solid tumor cells are thought to directly contribute to respiration malformation in the cancer cell. The consistency of distressed damage via constant reactivation /regeneration of oxygen species within the tumor cell's mitochondria makes it resistant to the effects of anti-cancer drugs, and by extension increases the electron transport dependence by amplifying respiratory malfunction.

The Theory of altering drug sensitivity via nanotechnology for treating solid tumors with respiratory deficiency / hypoxic conditions was first established using an in vivo experimental protocol . The protocol was designed to examine the effects of mitochondrial defects and hypoxia via an intrusion of nanotechnology bases cancer drugs .The protocol assessed the two major factors underlying the Warbury effect on cellular sensitive hypoxic micro environment cancer agents.

Rui Hua xu etal. created an experimental model by cloning human lukemia cells (HL-6) and human lymphoma (Raji) cells. The cells were used to assess mitochondrial respiratory defects via differential damage to the mtDNA of the cells. The experimental procedure saw the synthesis and use of BrPa based nano-prodrugs on human lukemia and lymphoma cells invivo. The cells were impregnated to promulgate tumor growth within a mouse after which the BrPa produg was inculcated and observed over a 24 day period. The biochemical and molecular characterization of the cell growth were assessed using the FACSCalibur flow cytometer with cell quest software. To assess the effects of the nano-drugs on the tumor cells transmission electron microscopy was employed. TEM analysis revealed abnormal swelling of the mitochondria in the leukemia cells, it also indicated a pale matrix with disorganized cristae. Biological analysis also indicated little or no oxygen uptake by the cells indicted little or no mitochondrial respiration occurring.

5. Conclusion

We have seen great progress in the area of nano-medicine modulation and enhanced cancer therapy in recent years, however many challenges remain towards solid tumor Hypoxia and clinical impacts of those innovative strategies being tested. The understanding of how hypoxia promulgates tumor resistance to drugs has attracted and sparked investigators and subsequent research has ensued .These numerous perspectives and methods shows promising potential as discussed in the review and gives us a fighting Chance at eliminating cancers in the near future .

It can be noted that one of the most crucial requirement for hypoxic targeting modalities is the development of improved predictive technologies for patient diversification. These modalities will not only evaluate hypoxic dependency but will also determine the sensitivity of each solid tumor formations. All solid tumor host genetic markers that can be explored to revolutionize the matching of hypoxic -targeting nano drug based therapeutics with patient individuality in mind. Nano-based drugs have the potential to create clinical niche for tumor cell diversification within the hypoxic sensitive tumor category, it is believed that this will intern create a functional rational for further clinical development.

With that said, the advancement in the field of nano-medicine remains so as to clarify how the use of nano-medicine will create a solid foundation in developing the perfect mechanism in combating solid tumor hypoxia and drug resistance by extension.

6. Conflict of Interest

There is no conflict of interest in this review.

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