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Increasing the Clinical Efficacy of Radiotherapeutics for Solid Tumors by Inhibition of Hypoxia Inducible Factor-1a

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Abstract: Radiotherapy is commonly used in a variety of tumor types and is effective in control of long term progression and may be curative under certain circumstances. Radiotherapeutics have been shown to be the single most effective therapeutic for cancer and only account for 5% of the total cost. However, treatment of cells with radiation causes the tumor microenvironment to undergo changes and leads to cycles of hypoxia inside the tumor which allows for the cell to undergo angiogenesis, metastasize and may lead to repopulation of the tumor. Current systemic chemotherapeutics typically have an additive toxicity due to the unintended targeting of non-cancerous cells. A nanoparticle conjugated with the iRGD peptide is proposed to allow for selective uptake by the CendR pathway in the tumor tissues. A systemic selective chemotherapeutic agent that will inhibit hypoxia inducible factor-1, preventing angiogenesis, that is co-administered with radiotherapy may increase the clinical efficacy of the radiotherapeutics and may have the greatest impact on currently available cancer treatment.

Introduction

Radiotherapy (RT) is used as a treatment option for approximately 50% of all cancer patients. RT has shown to be effective in the control of the long-term progression of tumors and in a substantial proportion of cases can be curative (1). According to the National Cancer Institute, there are two main forms of radiotherapy, internal radiation therapy and external beam radiation. Internal radiation therapy requires the source of radiation to be put within one's body. The form of radiation can either be solid or liquid. Internal radiation therapy with a solid source is termed brachytherapy and is a form of local treatment, meaning it treats only a specific part of your body. Internal radiation with a liquid source is called systemic therapy. This form of radiation will be transported in the blood to tissues throughout the body, where it will target and kill cancer cells. External beam radiation therapy is a form of local treatment that is accomplished by focusing a beam of radiation on the tumor (2).

Radiotherapy has been shown to activate a connected series of processes in tumor microenvironments including: inflammation, cycling hypoxia, revascularization and extracellular matrix remodeling (ECM) (3). Radiotherapy can cause cell death by inducing DNA damage. The induction of DNA damage is caused directly or indirectly by free radicals which are generated from the radiolysis of water. In the presence of oxygen, O_2, generation of reactive oxygen species occurs and acts as a positive feedback loop, further enhancing DNA damage (4).

Combining RT with a systemic treatment option, either with a biologically targeted chemotherapeutic or a cytotoxic chemotherapeutic to further enhance RT has had extensive interest in clinical research. A combinational therapy option would be beneficial over a prolonged course of RT due to the time it takes for DNA damage to cause cell death (5). One example of a current combination therapy option is the use of Cisplatin with RT which has shown to be beneficial for: head and neck, lung, esophageal, cervical, and bladder cancers (6).

This form of combinational therapy has shown to increase the clinical efficacy of RT, compared to if RT or cisplatin were used alone (7). However, Cisplatin has the ability to arrest cells in the G2 phase of the cell cycle, and when it targets noncancerous tissues, it leads to an additive toxicity (7).

Due to this fact, research into systemic chemotherapeutics that will not lead to additive toxicity is necessary. In this literature review, the idea of using a nanoparticle that will induce inhibition of HIF-1α as a form of a biologically targeted systemic chemo-therapeutic agent along with RT to increase the clinical efficacy of RT is looked at as a possible new therapeutic approach.

The tumor microenvironment

Solid tumors are not only composed of malignant cancerous cells but also include a collection of stromal cells that are related to: angiogenesis, inflammation, and growth of fibrous or connective tissue (8). This creates an atypical ECM that harbors cells including: fibroblasts, immune cells, red blood cells, epithelial cells, and endothelial cells. The cancerous cells take advantage of the densely packed ECM via mutations to create a harsher environment that supports tumor progression and treatment resistance.(9) This environment is thought to arise during the rapid proliferation of the cancerous cells. During this state of tumor development, the cells use vast amounts of nutrients and oxygen from normal vasculature which quickly leads to a hypoxic state, an oxygen deprived state, and a nutrient deprived state inside the solid tumor (10).

The hypoxic state leads to the activation of the hypoxic pathway through the activation of the hypoxia inducible factor 1 (HIF-1). HIF-1 is a heterodimeric protein consisting of 2 subunits, HIF-1α and HIF-1β. Both subunits are composed of basic helix-loop-helix proteins, and when combined act as a transcription activator for the subset of HIF-1 targeted genes (10). HIF-1β is a member of the Aryl hydrocarbon nuclear translocator family, and is only found in the nucleus (11). Meanwhile, HIF-1α under normal oxygen conditions is found in the cytosol and undergoes rapid degradation (9).

HIF-α degradation is associated with the enzyme proline hydroxylase. Under normal oxygen conditions, HIF-α is transcribed and translated at a high rate but almost immediately undergoes degradation. Proline hydroxylase is responsible for the oxidation of 1 or 2 proline residues of HIF-1α to hydroxyproline. After conversion to hydroxyproline, pVHL will bind to HIF-1α, and catalyze the addition of numerous ubiquitin molecules to the protein. Ubiquitin acts as a biological tag, and will signal for HIF-1α to be degraded in a proteasome (9).

In oxygen deprived conditions, proline hydroxylase fails to oxidize the proline residues. This allows for HIF-1α to evade degradation, allowing for the protein to translocate to the nucleus where it binds with HIF-1β and forms the heterodimeric protein leading to its activation (1). In previous studies, it has been found there are three

variants to the HIF heterodimeric protein which contain variant α subunits. These variants are termed as HIF-1α, HIF-2α and HIF-3α (12). HIF-1α and 2α have been shown to act similarly except in rare occasions of overexpression of the α-subunits in different cancer subtypes. HIF-3α has actually been shown to inhibit HIF-1α and HIF-2α (12). In this article, the focus is on the variant HIF-1α and how it leads to changes in the tumor microenvironment during hypoxic states inside solid tumors.

Disarrayed vasculature

Cytokine signaling for activation of endothelial cells during cycles of hypoxia inside solid tumors is known to cause formation of new vasculature by a process called angiogenesis (13). A hallmark regulator of angiogenesis is the hypoxic pathway. Upon formation of the heterodimeric transcription factor HIF-1, gene transcription for over 200 genes becomes induced. Of these 200 genes, which mostly are poorly understood, there are three main pro-angiogenic factor genes known to induce angiogenesis: Vascular endothelial growth factor (VEGF), platelet-derived growth factor (PDGF), and transforming growth factor-α (TGF-α) (9). VEGF will induce the recruitment and maturation of endothelial cells to form new capillaries within the solid tumor (14). PDGF stimulates mesenchymal cells, such as fibroblasts and pericytes which are associated with endothelial cells and help in the formation of new capillaries. TGF-α stimulates the recruitment and maturation of many types of cells to the tumor ECM including epithelial cells (9).

When forming new vasculature, first there must be some degradation of the existing ECM to create space for the formation of the new vessels. Matrix metalloproteinases (MMPs) are responsible for this degradation. MMP-2 is known to be directly expressed upon activation of HIF-1 (11). In the ECM, after degradation occurs, newly recruited endothelial cells will migrate to the area and form new endothelial tubes. These endothelial tubes are the primary structure of new capillary beds. Branching of these tubes is known to arise during modulation of Notch signaling. HIF-1α is responsible for the modulation of the Notch signaling by directly binding to the Notch intracellular domain. Newly recruited endothelial cells will begin forming tubes that extend from existing vascular network on the leading edge. After formation of these newly formed tubes, the immature endothelial cells will recruit vascular supporting cells, which have already been recruited to the tumor microenvironment by PDGF. The supporting cells include pericytes and smooth muscle cells that will form the basement membrane around the endothelial tube, leading to the formation of new vasculature and capillary beds. The final step in the formation of the vasculature is accomplished by fibronectin. Expression of fibronectin has been shown to be directly regulated HIF-2α in hypoxic states (11).

New vasculature formation is disarrayed when compared to that of normal tissue. Upon modulation of Notch signaling, by HIF-1α, extensive branching occurs in the newly formed vasculature. In normal tissue, branching of the vasculature is under tight regulation. Every cell needs to be within a certain distance, approximately 40 microns between adjacent capillaries, to ensure adequate oxygen and nutrients are available for the cells. Typically, in most living tissue there is only 1 to 3 cells that span the width of a capillary bed(15).

In solid tumors, it has been shown that this branching is more extensive, due to the rapid proliferation of the tumor cells. Rapid proliferation means the tumor will have a higher metabolic demand, which leads to the production of excessive pro-angiogenic factors. Also, tumor vessels are continuously being remodeled inside solid tumors, leading to endothelial cells losing contact with the basement membrane, causing the capillary beds to rupture. The rupturing of capillaries causes the vessels to have larger pores and become leaky (16).

Tumor hypoxia effects

The network of vasculature that is formed during cycles of hypoxia inside tumors is known to cause an increase in interstitial fluid pressure (IFP) and solid tissue pressure (STP) due to the rupturing of capillaries and the lack of formation of lymphatic vessels inside solid tumors. In most solid tumor animal models, IFP will increase between 10-100 mmHg (17). Due to the leakage the capillary beds, excessive leakage of proteins and fluids occur. This leads to an elevated microvascular pressure (MVP) and a decreased osmotic pressure compared to that of normal tissue. The fluid buildup in the tumor is a result of the lack of lymphatic vessels that normally uptake excess fluid present in the interstitial fluid (1). Due to these changes, there are implications for the delivery and uptake of nanoparticles into solid tumors.

Along with the buildup of fluid, the cancerous cells are undergoing rapid proliferation which leads to an increase in STP. As STP increases, there is a direct correlation with the collapse of vasculature. This leads to an increase in vascular blood flow resistance, resulting in a greater increase of IFP and MVP promoting the accumulation of more fluid in the interstitium of the tumor (1). Depending on the location in the tumor, IFP changes due to STP. In the center of the tumor, there is the greatest density of cells, leading to a high STP. This leads to a uniform elevation of IFP that will approach MVP. When IFP is near or equal to that of MVP, there is a limited transport of pharmaceuticals, oxygen and nutrients (1).

Near the edges of tumors, there is a decrease in STP because there is a lower density of cells. This means that there will be a lower IFP on the edges of tumors, but because it is higher than that of surrounding tissue, there is a possible outflow of fluid into surrounding tissue (1). This representation of the solid tumor microenvironment explains how the insufficient fluid flow works in favor of the tumor, allowing it to evade the immune

system by preventing antigen presenting cells from leaving the tumor and preventing the uptake of chemotherapeutics into the core of the tumor.

Individuals with solid tumors that have cycles of hypoxia are known to have poor prognoses (18). This is due to the positive correlation in cycling hypoxia leading to increased tumor aggressiveness and resistance to radiotherapy and chemotherapeutics (18). As shown previously, cycling hypoxia within solid tumors leads to the formation of new vasculature, that presents implications for the uptake of chemotherapeutics and certain radiotherapeutics.

According to the National Cancer Institute, tumor aggressiveness is defined by the ability of a tumor to form, grow, or spread quickly (19). For a tumor to spread, metastasize, the tumor cells will undergo an epithelial-to-mesenchymal transition (EMT). For an EMT to occur, there are a variety of transcription factors that need to be activated. Two of these transcription factors are TWIST1 and SNAIL1.

In a study conducted by Yang et al., it was shown that HIF-1α regulates the expression of TWIST1 upon binding to the hypoxia-response element (HRE) which is found in the proximal promoter of TWIST1 (20). This means HIF-1α is directly correlated to the ability of the tumor to promote metastasis leading to a worse prognosis. However, when they used siRNA-mediated repression of TWIST1 in an environment where HIF-1α was over expressed, the cells reversed the EMT and

metastatic phenotypes (20). This implicates the direct correlation of HIF-1α and metastasis, showing that TWIST1 is responsible for the EMT.

According to K.J. Wu, who created a method for predicting metastatic potential in head and neck squamous cell carcinomas (HNSCCs) that co-express: HIF-1α, TWIST1 and SNAIL1, there was more than a 90% probability of metastasis. Comparing that to HNSCCs that co-express TWIST1 and SNAIL1, not HIF-1α, only had a 50% probability of metastasis (21). Based on his predictions, HNSCCs that co-express all three proteins have a substantially greater probability of metastasis, leading to a poorer prognosis and patient survival rate.

TWIST1 is unique because not only does it help trigger an EMT, but it also has been shown to facilitate tumor cells to escape the senescence program found within every eukaryotic cell (22). The senescence program determines how many times a cell is allowed to replicate before it become senescent, the inability to proliferate, acting as a generational clock. In a normal cell, this is typically around 70 cell cycles. Upon completion of each cell cycle, the telomeres at the end of the DNA will be shortened (9). This means tumor cells expressing TWIST1 have the ability to replicate with telomere lengths that would normally trigger apoptosis.

When cells are allowed to replicate without a proper senescence program, there is an increase in genomic instability. This occurs due to chromosomal end-to-end

fusions that are called breakage-fusion-bridge (BFB) cycles. Telomeres normally prevent this from occurring by capping the ends of chromosomes. When chromosomes become uncapped, such as in a cell without a proper senescence program, BFBs cause the fusion of sister chromatids and random breaks, which may cause new mutations to occur (23). The formation of new mutations in the DNA of the cancerous cells may increase tumor aggressiveness by inducing changes that increase the ability of the cancerous cell to proliferate, grow, or to metastasize.

Effect of radiotherapy on the tumor microenvironment

Radiation therapy is known to kill cancer cells by directing high physical energy in forms of radiation onto the cancer cells. RT has been used as a cancer therapeutic since the late 19th century. Through years of research, numerous advancements have been made in the clinical efficacy of RT (2). As we gain a better understanding of the molecular mechanisms in treatment sensitivity and resistance, these advancements have been made possible. RT is most commonly used in the treatment of localized malignant tumors during the progression of cancer. Recent studies have shown that radiotherapy is the single most cost-effective cancer therapeutic and accounts for only 5% of the total cost of cancer care. Due to this fact, increasing the clinical efficacy of RT would benefit the largest number of patients (5).

Tumor cell death

Radiotherapy directly causes tumor cell death, resulting in the reduction of the cell population by numerous mechanisms. Ionizing radiation can act on the cancer cells by causing direct damage to the cell membranes leading to cell lysis (5). Also, ionizing radiation can lead to the production of free radicals which are produced by the ionization or excitation of water within the cells. These free radicals can cause direct DNA damage, which will not cause direct cell death, but if not repaired, will lead to cancer cell death. The most lethal forms of DNA damage will induce double stranded breaks which will result in cell death if not repaired adequately. However, if the cancer cell is able to adequately repair the DNA damage, the cell will be able to survive and increase their genomic stability (5).

During the course of a multi-day course of RT to a radio-responsive tumor, the total mass of the tumor will be decreased, leading to a decrease in the tumor's production of pro-angiogenic cytokines, which causes the endothelial cells making up the vasculature to destabilize, leading to the regression of the vasculature (1). This will ultimately lead to the tumor to have a decrease in blood supply, lowering the availability of nutrients and oxygen and will trigger the portion of solid tumor that is still intact to become hypoxic. Activation of the hypoxic pathway has been well known to occur following radiation treatments.

Effects of hypoxia pathway post-treatment

Angiogenesis has been shown to be correlated with activation of the hypoxic pathway. Upon completion of treatment with RT, solid tumors have been shown to experience hypoxia. After RT, it has been shown that in solid tumors, the proangiogenic cytokines increase dramatically in the following 6-8 hours (18). This means, if the patient is undergoing RT every other day, by the beginning of the next round of RT, the tumor will have already undergone angiogenesis and may have possibly repopulated due to the resupply of nutrients and oxygen to the solid tumor.

Cancer stem cells (CSCs) are the main reason for tumor regrow after the completion of a course of RT. CSCs are fully capable of repopulating a tumor due to their ability to activate telomerase, an enzyme that increases the length of telomeres, giving the cells the ability to replicate without a senescent program. The majority of the cells that make up the human body do not have this ability. Thus, if CSC's arise, they play a key role in inhibiting an effective cancer treatment. Recent studies have provided evidence that suggests hypoxia promotes the persistence of stem cells (24). In a study by Gianluca D'Ippolito, mesenchymal cells were isolated and cultured at various levels of oxygen. It was found that under higher levels of oxygen, differentiation was more likely to occur. Also, when the PO_2 was decreased, mimicking a hypoxic environment, the stem cells were more likely to not differentiate and maintained their self-renewal capabilities (25).

CSCs have also been directly correlated with a solid tumor's ability to undergo metastasis, a process that was explained above to also be influenced by hypoxia. Stated by Richard Hill, "The potential role of hypoxia in enhancing EMT and stemness in cells raises the possibility that the adverse effects of tumor hypoxia might be partially explained by its dual effects of promoting EMT and the self-renewal of CSCs" (24). Treatment resistant, or commonly known as radio-resistant, cells provide an obstacle during the course of RT treatment. If radio-resistant cells are present in the tumor, the RT will be ineffective at clearing the totality of the tumor mass (1).

Nanoparticle based radio-chemotherapy

Nanotherapeutic specific for HIF-1α inhibition

Currently, a surplus of HIF-1α inhibitors have been created and designed. However, these inhibitors have been shown to have numerous downstream effects, leading to the creation of adverse effects when used as a systemic chemotherapeutic agent (26). Lack of targeting HIF-1α on the surface of cancerous cells has been an important obstacle in the creation of a systemic chemotherapeutic agent. Also, HIF-1 has numerous downstream affects due to its ability to upregulate numerous genes which increases its risk of additive toxicity (10). However, if we are able to specifically target the inhibition of HIF-1α only in cancer cells, there would be a decrease in adverse effects and would result in a systemic chemotherapeutic that would have an increased efficacy(3).

As described above, the vasculature in solid tumors is unlike that of normal tissue. The endothelial cells are more spaced out, creating larger pores, leading to the vasculature to be leakier. Due to this, it is proposed to design of a nanoparticle that would be larger than that of the pores of normal vasculature, which is in the range of 6-12 nm (27).

Delivery of nanotherapeutics to solid tumors is believed to be increased by administering the nanoparticle systemically. By creating a stable nanoparticle that has a long half-life, the chances of it actually reaching the vasculature and being transported into the solid tumor without crossing the normal vasculature wall will decrease adverse effects (27). Renal clearance of particles that are smaller than 6 nm is very rapid and clearance of particles in the spleen of a nanoparticle that is larger than 200nm will also increase (27). Based on these findings, a particle in the range of 12-200 nm would have an increase in half-life, thereby increasing the possibility that it would enter the solid tumor.

Nanoparticles that are currently in clinical trials are typically found to have a size within this range (27). The most common type of nanoparticle that has been tested in these clinical trials uses a liposomal formulation, which can be designed to be a specific size(28). By using a liposomal formulation, the nanoparticle delivery is believed to increase, and can be designed so that upon uptake into the tumor microenvironment, based on physiological characteristics such as pH, targeted release of the HIF-1α inhibitor could be achieved (28).

Specific targeting and the synergist effects of radio-chemotherapy

Specific targeting of solid tumors by a chemotherapeutic through the CendR pathway has been under extensive research. The CendR pathway involves a three-step process using a tumor targeting peptide (TTP) sequence leading to the formation of endocytic vesicles that are approximately 200nm (29). In the first step, an integrin-binding RGD (iRGD) sequence motif within the TTP binds to $\alpha v\beta 3$ and $\alpha v\beta 5$ integrins which are specifically expressed by tumor endothelial cells. Upon binding, the sequence is cleaved by an unknown protease leading to the activation of a second motif in the sequence, the CendR motif (R/KXXR/K). The CendR motif can then bind to neuropilin-1 and neuropilin-2, which activates the CendR endocytotic pathway (29).

The CendR pathway is most similar to macropinocytosis; however, unlike macropinocytosis, it is receptor dependent. The required receptor is the neuropilin receptor. Upon activation of the receptor, the endocytic vesicles will form, which can uptake any particle small enough into the cell such as a nanoparticle (30). The nanoparticle will then be released into the cytoplasm and is free to undergo its own specific activity. In this case, the activity would act as an inhibitor of HIF-α, which would prevent its translocation into the nucleus and prevent activation of the HIF transcription factor.

Along with the upregulated expression of the $\alpha v\beta 3$ and $\alpha v\beta 5$ integrins in the tumor endothelial tissue, the neuropilin

receptor is also overexpressed, leading to an upregulated activation of the CendR pathway (29). The receptor is upregulated due to the fact that tumors are typically in a nutrient deprived state. The enzyme mTOR acts a senor for nutrients, and when it senses a deprived state, it will signal for the overexpression of neuropilin (29). Neuropilin has been shown to be overexpressed in a wide variety of tumors including: prostate, breast, melanoma, pancreas and glioblastoma cancers (28). One advantage to this specific receptor is that it has not been shown to be upregulated on endothelial cells in normal vasculature. Due to this fact, peptides that bind to the neuropilin receptors are termed TTPs (28). Also, it has been found that drugs that are conjugated with the iRGD peptide have an increased accumulation in tumors by 4-5-fold. This was shown in mice models with solid tumors (31).

Currently evidence is strongest for the use of nanoparticles conjugated with the iRGD peptide. However, it is believed that liposomes may be able to be modified with the iRGD peptide allowing for the activation of this pathway. Liposomes are suitable for nano drug delivery due to their controllable size and their ability to be modified (28). The issue that arises with the use of liposomes is that they have been shown to not penetrate deep into tumors as well as nanoparticles due to the complexity of the highly dense ECM and the rapidly increasing IFP in the center and periphery of the tumor (28). Because of this fact, it is proposed at the time to further study the conjugate of a HIF-α inhibitor with the iRGD peptide, which will avoid the

obstacles that are present with the use of liposomes.

Increased clinical efficacy of radiotherapy can possibly be achieved with a targeted release of an HIF-1α inhibitor (4). By using a systemic chemotherapeutic such as the one proposed in combination with normal RT treatment could prevent the adverse effects that are seen upon activation of the hypoxia pathway due to RT treatment. Using this approach would prevent the formation of new vasculature within the solid tumor and could possibly cause necrosis of the tumor due to lack of access to adequate nutrients and oxygen. With the hypoxia pathway already inhibited, the tumor would not be able to undergo angiogenesis and could possibly prevent future metastasis from occurring, which both have been shown to be upregulated by HIF-1 formation.

Increased efficacy for cancer-type specific chemotherapeutics would be beneficial if there are CSCs present in the solid tumor. CSCs are responsible for the regrowth of new tumors and must be eliminated to prevent future relapses (24). Upon completion of a round of combined RT with a specific HIF-1α inhibitor, the solid tumor will have decreased blood flow, decreasing the chances of the chemotherapeutic from reaching the CSCs. However, if there are CSCs present that are radio-resistant, research has shown, the best treatment option typically relies on the use of present systemic chemotherapeutics (24). Since there will be a decrease in the overall mass of the solid tumor, the systemic chemotherapeutics could possibly have a better chance of targeting the CSCs, which would lead to the increased efficacy. This is outside the scope of this paper, but it may benefit from future research upon successful formulation of the combined RT, HIF-1α treatment plan.

Conclusion

Radiotherapeutics have been shown to be an effective treatment option for a variety of tumor types in controlling tumor progression and may being curative under certain circumstances. Although radiotherapy is effective, it has been shown to cause changes in the makeup of the tumor microenvironment leading to cycles of hypoxia. Although theses cycles have no initial impact on the tumor, they may lead to downstream effects such as angiogenesis, tumor proliferation and tumor metastasis, which are correlated with a worse prognosis. These effects have been shown to be correlated with the activation transcription factor HIF-1 during a hypoxic state. Activation of the transcription factor occurs when HIF-1α subunits translocate from the cytoplasm into the nucleus and bind with the HIF-1β subunits and leads to an increase in the expression of VEGF, PDGF, and TGF-α along with a variety of other genes.

When angiogenesis occurs in the tumor microenvironment, the vasculature is disarrayed compared to that of normal vasculature due to the overexpression of pro-angiogenic factors. The vasculature is leakier leading to the accumulation of excessive fluid and proteins inside the tumor. Also, as the cells deep within the tumor are proliferating, STP increases due to the increase in cell density. Together, this leads to an increase in the IFP which has been shown to hinder the distribution of chemotherapeutics deep in the tumor.

Tumor targeting peptides, which express a iRGD motif, selective for endothelial cells within the tumor have shown promising in mice models to increase the accumulation of nanoparticles deep within tumors upon activation of the CendR pathway. A nanoparticle that can inhibit the translocation of HIF-α subunits into the nucleus that is conjugated with a iRGD motif may prevent the accumulation of excess pro-angiogenic factors in the tumor microenvironment post-treatment by radiation. Therefore, this nanoparticle could potentially increase the clinical efficacy of radiotherapeutics and being selective to only tumor tissue would decrease the risk of additive toxicity.

Limitations

HIF-1 has been highly correlated with the accumulation of pro-angiogenic factors; however, they are not the only transcription factors that can signal for their overexpression. Therefore, although HIF-1 inhibition may successfully prevent angiogenesis, cell growth/proliferation and metastasis, there may be other pathways that the cell could activate to accommodate the revascularization of the tumor (9).

Future Research

This is a proposal of a new conjugated nanoparticle that has yet to be made or tested in a laboratory or clinical setting. Thus, research needs to be completed in the creation and efficacy of the proposed selective chemotherapeutic and on how specifically it is taken up by the tumor, how

much it may decrease the accumulation of pro-angiogenic factors and if the cancerous cells will activate other pathways to revascularize the tumor.

Acknowledgments

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Appendix A

Formatting of this paper was completed following the National Library of Medicine (NLM) guidelines.

Appendix B

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