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Repurposing of Aspirin to Regress Tumor from its 'Root' – The Cancer Stem Cells

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Abstract

The role of aspirin as a Non-Steroidal Anti-Inflammatory Drug (NSAID) is well established and its usage is prevalent since several years. Currently, due to the time lag and high cost required to final public release of any new drug from the initial perception of its development, ascertaining new uses for FDA-approved pre-existing drugs to provide the quickest possible transition from bench to bedside is on the rise. Such effort, in effect, would benefit both the patient and the pharmacological companies, as the process would be faster and cheaper than the development of a completely new drug. In this context, the effect of aspirin in pathological conditions other than inflammation has been explored. Aspirin, as a prophylactic agent, has been documented to lower the incidence of secondary cardiovascular attacks in cardiovascular disorders. Overwhelming evidence in large number of patients for different time intervals indicates that aspirin possesses anti-cancer activity and the potential to prevent different cancers. Recently, there is a paradigm shift in the understanding of carcinogenesis. In this regard, self-renewing Cancer Stem Cells (CSCs) or tumor initiating cells within a tumor cell population have been endowed with the responsibility of tumor initiation, development and progression. CSCs are spared even after chemotherapy and responsible for tumor relapse. Targeting these highly drug-resistant CSCs is, therefore, of utmost necessity to achieve radical cure in cancer. Interestingly, aspirin has been reported to sensitize CSCs towards conventional chemotherapeutic drugs by modulating the molecular architecture of these cells. Repurposing of aspirin in regressing tumor from its root – the cancer stem cells – is therefore a major boost not only to the patients but also to the pharmaceutical industry.

Keywords: Angiogenesis; Apoptosis; Aspirin; Cancer Stem Cells; Metastasis; Repurposing

Abbreviations

NSAID: Non-steroidal anti-inflammatory drug; FDA: Food and Drug Administration; CSC: Cancer Stem Cell; IARC: International Agency for Research on Cancer; WHO: World Health Organization; COX: Cyclooxygenase; VEGF: Vascular Endothelial Growth Factor; Sp: Specificity Protein; TRAIL: TNF-related Apoptosis Inducing Ligand; ALDH1: Aldehyde Dehydrogenase 1; PDA: Pancreatic Ductal Adenocarcinoma; NSCC: Non-Stem Cancer Cells; PKC: Protein Kinase C; EMT: Epithelial to Mesenchymal Transition; TIC: Tumour Initiating Cell; TACE: Trans-Arterial Chemo-Embolization.

Introduction

Repurposing of drugs: old wine in new bottle

From the initial perception of the development of a new drug, i.e., designing, synthesis, characterization, testing the functionality and toxicity, to public release is a very slow and costly process. The process of drug development is an arduous and time consuming task where the identification and formulation of new drugs had not been able to keep pace with the amount of resources put into it. According to Paul et al., drug discovery and its delivery to market take more than 10 years with an estimated cost of ~$1.8 billion [1]. In addition, the success rate is <10% with high possibility of late stage failures that account for additional cost thereby making the patented drugs usually very expensive. The process involves the rejection of various prospective candidates due to the stringent mode of scrutinization. To circumvent this growing disparity between the growing need of new drugs and the trickling down pace of discovery of new drugs, the process of drug repurposing is gaining much importance. The initial impetus in this field came after the landmark article by Ashburn and Thor in 2004, which put forth into the concept of using an established drug for another clinical purpose. They emphasized on the need to find usefulness of the existing drugs beyond their established medical usage [2].

On the other hand, according to the International Agency for Research on Cancer (IARC), part of the World Health Organization (WHO), mortality due to cancer is more in poor countries than AIDS, malaria and tuberculosis combined together [3]. Therefore, the current need is the requirement of new anti-cancer drugs which could liberate the masses from its deadly clutches. But discussed above the process of discovery and identification of new anti-cancer drugs would be both time consuming and probably not commercially viable as large section of the masses would simply not able to afford such a costly drug. From the point of view of pharmaceutical companies, the identification of new anti-cancer function of the existing drugs would be a better option. In this regard, repurposing of approved drugs could make way for the rapid possible transition from bench to bedside.

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This concept has already been approved by the US Food and Drug Administration (FDA) and has attracted a lot of attention recently [4-6]. The major advantage of repurposing any drug is that from years of its effective use in clinical studies, there already exists valuable information about its optimum dose regimen, pharmacokinetics, pharmacodynamics, metabolic profiles and side effects. However, for repurposing, validation of the connection(s) between the drug already-in-use and new molecular targets is of utmost necessity for the development of the new therapeutics from the old drug. Despite these precincts, repositioning of drug reduces time, cost and risk of failure. Therefore, repurposing of drugs with indications other than oncology has emerged as a promising approach, either alone or in combination therapy with different drugs, for a disease like cancer for which despite all the efforts, relapse-free survival of patients is still a 'dream not fulfilled'.

Aspirin: Can this old drug play new tricks?

Acetylsalicylic acid, a Non-Steroidal Anti-Inflammatory Drug (NSAID), also known as aspirin was first synthesized by Felix Hoffmann at the Bayer Company in 1897, was subsequently used as an anti-pyretic, anti-inflammatory and analgesic drug [7]. This FDA-approved drug was originally used to treat arthritis, odontalgia and other pains elicited by inflammation. However, other pharmaceutical benefits of this drug were recognized with time. Studies exhibited that aspirin has the potential to inhibit the aggregation of platelets thereby helping patients with atherosclerotic plaques which are susceptible to clot formation when chunks of plaques are formed or dislodged from its original locus to a new one [8]. The role of anti-coagulants like aspirin is indispensable as an artery blockage could lead to oxygen deprivation which could ultimately lead to myocardial infarction, if the blockage is in the cardiac tissue. NSAIDs like aspirin are known inhibitors of cyclooxygenase (COX) enzymes. In platelets aspirin induces acetylation of COX that ultimately aids the anti-thrombotic effect [9]. Along with the anti-coagulant effect, aspirin acts as a prophylactic agent against conditions which range from heart disease and stroke to cancer. Patients who had encountered any cardiovascular disease in the near past have a high risk of experiencing cardiovascular events like myocardial infarction or ischemic stroke. In such cases, if the patients are administered aspirin, there exists a lesser chance of encountering any secondary cardiovascular events [9]. It was revealed that acetylation of COX by aspirin occludes the production of thromboxane in platelets; further preventing their aggregation and ultimately resulting in the relaxation of vascular wall [10]. Apart from the anti-platelet action, it also inhibits plaque formation by enhancing TGF-β production [11]. Thus, the discussion reveals that aspirin has been extensively identified for preventing cardiovascular diseases. Further, several research groups have carried out trials which showed that administration of aspirin resulted in a marked reduction in non-fatal infarction and stroke. The low dose regimen of aspirin was revealed to be as effective as high dose regimen. Moreover, the long-term use of aspirin was associated with additional mortality benefits [9]. Furthermore, it is well known that the onset of diabetes causes an increase in the occurrence of cardiovascular diseases in patients. Due to the increase in the prevalence of coronary thrombosis in the patients suffering from diabetes, there is 2 to 4-foldrise in cardiovascular disorders [12]. In the diabetic patients, aspirin demonstrated a substantial reduction in the risk of occurrence of both fatal and non-fatal myocardial infarction [13]. In effect, majority of the recognized health organizations including AHA/American Stroke Association have recommended the administration of aspirin for prevention of cardiovascular disorders [9]. Therefore, the above discussion focuses on the use of aspirin for other diseases apart from its conventional use. This further stems the discussion whether it is effective against cancer. The following sections will discuss the effectiveness of aspirin for treating cancer.

Aspirin: A New Addition to Anticancer Therapy

Inflammation and cancer: A deadly liaison

Cancer has taken the place as one of the most prevalent non-communicable diseases of the current times. According to the World Cancer Research Fund in 2012 there were approximately 14.1million cancer cases throughout the world. By the year 2035, it is estimated that the figure could rise up to 24million [3]. Apart from this, through decades of research inflammation has emerged as an essential constituent in the development of cancer. Chronic inflammation facilitates the favorable environment necessary for stimulating tumor development. Inflammatory mediators in the tumor microenvironment aid the neoplastic process. Therapeutic approaches involving anti-inflammatory drugs are hence gaining importance [14]. Therefore, aspirin being an anti-inflammatory drug might serve as an anti-cancer agent by modulating the inflammatory mediators involved in carcinogenesis. Consequently, current research on unveiling new pharmaceutical benefits of aspirin have depicted an interesting aspect showcasing aspirin intake correlates with reduced onset of some types of cancers [15]. In the case of CAPP2 trial, volunteers who were administered aspirin had a 63% reduction in the relative risk of developing colorectal cancer compared to those provided with placebo [16] Another study by Cao et al. [17] showed that the long-term use of aspirin for 6 years or longer showed 19% decrease in the risk of colorectal cancer and 15% reduction in the onset of gastrointestinal cancer. Similarly, aspirin usage is associated with diminished risk of breast cancer death [18]. Therefore, these studies indicate the possible use of aspirin against cancer. However, it is essential to unveil the underlying molecular mechanisms in order to get a clear picture of the anticancer effect depicted by it. As previously stated that aspirin is a COX-inhibitor, it probably prevents the onset of carcinogenesis through both COX-dependent and COX-independent mechanisms [19]. Inflammatory conditions elevate COX-2 levels which is an indicator for melanoma progression [20]. Therefore, the inhibition of COX-2 by aspirin might reduce the incidence of melanoma in humans. The use of aspirin resulted in an 11% reduction in the risk of melanoma in patients and 30% reduction when the intake of aspirin was more than 5 years [21]. Besides COX inhibition, Aspirin is found to inhibit the activity of NFκB (a transcription factor) which is associated with the activation of numerous essential genes. One of the functions of NFκB is to promote the inflammatory factors as well as to reduce the progression of apoptosis. Therefore, this provides an alternative path or a COX-independent mechanism to check the progression of cancer by aspirin [22].

Aspirin in inducing apoptosis of cancer cells

Numerous research investigations essentially highlight the ability

of aspirin to induce apoptosis in cancer cells. Different molecular mechanisms prevail by which the cancer cells are sensitized or killed by aspirin treatment. The earliest reports hint at translocation of Bax to mitochondria by aspirin releasing cytochrome c from mitochondria thereby triggering the apoptotic cascade [23]. Further studies emphasized that aspirin treatment impedes proteasome function which might ultimately lead to alterations in the mitochondrial membrane potential followed by cytochrome c release and inhibition of NF κB activity [24]. Apart from the intrinsic apoptotic pathway, aspirin also triggers apoptosis via extrinsic pathway involving FasL/Fas [25]. Redlak et al [26] demonstrated the involvement of Protein Kinase C (PKC) signaling in aspirin induced apoptosis wherein the two isoforms of PKC i.e., PKC α and PKC βII were overexpressed upon aspirin exposure. In a different study, aspirin mediated the induction of apoptosis in colorectal cancer cells by sensitizing them to TNF-Related Apoptosis Inducing Ligand (TRAIL), thereby enabling negation of TRAIL resistance [27]. Few other molecular mechanisms which mediate aspirin-induced apoptosis involve downregulation of Specificity Protein (Sp) Transcription Factors such as Sp1, Sp3 and Sp4 [28] and overexpression of calpain gene [29]. Above discussion signifies the role of aspirin in inducing apoptosis in cancer cells by modulating their molecular architecture.

Anti-angiogenic potential of aspirin

Neo-angiogenesis/neovascularization or new blood vessel formation is a vital step in tumorigenesis since the proliferating tumor mass essentially requires supply of crucial nutrients in order to support its growth. Hence, therapies are being developed to target angiogenesis [30]. In relation to this, several reports have provided clues that aspirin exhibits anti-angiogenic effects in a COX-independent manner [31]. In effect, it alters the level of angiogenic proteins such as Vascular Endothelial Growth Factor (VEGF) [32]. The expression levels of VEGF-A and VEGF-C declined significantly upon aspirin treatment following which the tumor development in murine sarcoma model was delayed notably due to the inhibition of angiogenesis [33,34]. These reports signify the potential of the anti-inflammatory drug aspirin in inhibiting tumor angiogenesis.

Aspirin hindering tumor metastasis

The lethality of the tumor mass is determined by its ability to metastasize and to proliferate at a new location. In the process of acquiring the metastatic ability the cancerous cells undergo Epithelial to Mesenchymal Transition (EMT), where the cells lose their distinct polarity as well as the cellular junctions are disrupted and the cells attain a distinct morphology [35]. One of the main indicators of EMT is the switching of E-cadherin to N-cadherin [36]. Moreover, inhibition of the expression of E-cadherin by the transcriptional modulator Slug, is essential for the progression of EMT [37]. The study carried out in our laboratory by Khan et al [38], demonstrated that aspirin represses Slug expression by blocking the activation of p65 subunit of NFκB, thereby hindering its translocation to the nucleus [38]. As a result, administration of aspirin aggravated the levels of E-cadherin and subsequently suppressed the levels of N-cadherin. This study therefore, provided the basis for the use of aspirin as an inhibitor of Slug which subsequently checks the progression of EMT and metastasis. Furthermore, in a recent report aspirin was found to inhibit metastasis along with angiogenesis by inhibiting the enzymatic activity of heparanase [39]. Additionally, the inhibition of COX-1 in platelets of mice by aspirin administration impeded the metastatic potential of the tumor cells [40]. The anti-metastatic potential of aspirin has been clearly evident from the above discussion.

The aforementioned sections essentially highlight the anti-cancer effect of aspirin giving enough clues for repurposing it for the same. However, it is important to note that there are thousands of drugs which exhibit anti-cancer effect but fail to eliminate the deadly disease. The solitary reason behind this failure is their inability to target the Cancer Stem Cells (CSCs) which are the master regulators of the disease. The following sections will highlight the role of these master regulators and further discuss the effectiveness of aspirin in sensitizing or targeting them.

Cancer stem cells – the sovereign regulator of tumor population

Despite extensive research explorations in the field of cancer biology, this detrimental disease remains unconquered. The failure of chemotherapy due to increased resistance is an emerging problem which impedes cancer treatment. Hence, investigations pertaining to tumor resistance and subsequent relapse are a realm of active interest. In the recent past, relevant studies in this field corroborate the Cancer Stem Cell (CSC) theory, which has helped gain a better understanding of the complex disease [41-43]. Among the various factors involved in the initiation of cancer, CSC theory is gaining immense acceptance. The CSCs are also popularly known as Tumor Initiating Cells (TICs) which have the potential to generate assorted cell types in the tumor mass [44]. The minimal functional definition of TICs refer to the cells which are capable of forming tumors *in vivo* with as low as 200 cells [45,46]. The existence of a CSC was primarily discerned by Bonnet and Dick [47] following this crucial breakthrough, existence of CSCs was recognized in different solid tumours [42]. This subpopulation is primarily responsible for governing the different aspects of initiation, progression and maintenance of tumours. Apart from this, tumour heterogeneity has been largely defined by the presence of CSCs which have the ability to self-renew and differentiate into multiple lineages [48]. This subpopulation of cell also immensely contributes to drug resistance followed by tumour relapse. Even though they manifest stem cell properties, they do not essentially emanate from the transformation of normal tissue stem cells [49]. Additionally, the self-renewal ability of the CSCs is dysregulated when compared to normal tissue stem cells. Asymmetric cell division is often used by normal tissue stem cells in order to sustain tissue homeostasis [50]. This mechanism is tightly regulated and any aberration may lead to dysplasia [51]. CSCs, unlike Non-Stem Cancer Cells (NSCCs), divide asymmetrically which helps to maintain the stem cell population along with the generation of progenitor cells capable of producing different cancer subtypes. Thus, they help repopulate the tumor mass. Further, the balance between symmetric and asymmetric division in CSCs is deregulated. CSCs associated with early stage tumors predominantly show evidence of asymmetric division while late stage tumors significantly suppressed asymmetric division and enhanced symmetric division [52]. Numerous markers such as CD44, CD133, EpCAM etc have been identified for isolation of CSC enriched populations from multifarious tumours. Breast CSCs are mainly characterized by the presence of CD44+/CD24-/low marker along with the overexpression of aldehyde dehydrogenase 1 (ALDH1) [53]. On the other hand, CD133 is a predominant CSC

marker in glioblastoma [54] while ovarian CSCs are characterized by CD44, CD117, and ALDH1 activity along with CD133 [55,56]. CD 44, CD24 and CD133 are putative CSC markers for colon and prostate cancers as well [57,58]. Numerous reports have highlighted the potential role of CSCs in cancer metastasis [50]. CSCs have been reported to be predominantly responsible for initiating the process of metastasis. Velasco-Velázquez et al [59] have reviewed the potential role of breast CSCs in metastasis. CSCs have been described to be the root of metastatic lesions along with the involvement of the microenvironment, thereby providing a stemness-metastasis link. Recently, it has been documented that tumors with high expression of stem cells and Epithelial to Mesenchymal Transition (EMT) markers with having low expression of claudin-3, claudin-4 and claudin-7 were associated with low rate of overall survival [60,61]. With the advent of CSC theory, it is increasingly becoming comprehensible that the phenotypes executed by tumor mass are driven by these CSCs. In line with this, CSCs have an immense potential to evade apoptosis in response to cellular environmental stimuli. The anticancer ability of the majority of chemotherapeutic agents is imparted by induction of apoptosis. In such a scenario, the resistant CSCs pose a major hurdle in cancer treatment [62,63]. Targeting these master regulators is thus, is of prime importance in order to achieve relapse-free survival of patients.

Is Aspirin Capable of Targeting Cancer Stem Cells?

Aspirin regresses tumor from its 'root' – the cancer stem cells

Epidemiological surveys have distinctly depicted the association between aspirin intake and reduction in cancer mortality. Several experimental studies have also emphasized on the potential benefits of aspirin as an anticancer agent [64,65]. With the emergence of the CSC theory, there is a growing need for developing therapeutic agents which can potentially target them. The essential question that arises here is whether aspirin can be used to target the CSCs. In this context, experimental studies demonstrated that aspirin not only inhibited the growth of breast cancer cells but also downregulated the self-renewal ability of the breast CSCs notably. Alongside it also inhibited the tumor outgrowth in xenograft mouse model by fostering apoptosis. Aspirin further, arrested the migration of cells in tumor xenografts and also assisted the reversion of EMT reprogramming by up regulating the epithelial markers E-cadherin and Keratin 19. Molecular insights revealed that aspirin showcases tumor inhibitory properties by impeding the TGF-β/SMAD4 signaling pathway [66]. It also abrogates the NFκB–IL6 signaling pathway which is responsible for CSC self-renewal [43]. Consequently, Zhang et al [67] demonstrated the ability of aspirin to thwart CSC characteristics in Pancreatic Ductal Adenocarcinoma (PDA) by downregulating ALDH1, thereby inhibiting spheroid formation. It also suppressed the CSCs derived from PDA patient tumors. Similar findings were also obtained in case of colorectal cancer where aspirin inhibited colospheres [68]. Furthermore, aspirin is also capable of attenuating the transcriptional activity of beta-catenin/TCF complex [69]. The essential role of beta-catenin in modulating CSC characteristics is well established [70-72] and hence targeting it using aspirin may potentially help eliminate the CSCs. Aspirin enhanced phosphorylation of protein phosphatase 2A [73] as well as beta-catenin [74] which curtailed the activity of Wnt/beta-catenin signaling pathway. In a similar fashion, aspirin is known to inhibit NF-κB [75] which has a crucial role in regulating CSCs. NF-κB signaling pathway is constitutively activated in CSCs boosting their survival [76-78] and hence, is a promising therapeutic target. Thus, aspirin has the ability to target multifaceted signaling mediators which may in due course reduce the CSC characteristics. However, the question arises whether it is capable of impeding the master regulators single-handedly? Research findings hint at better therapeutic strategies by using aspirin in a prodrug form or if used in combinatorial therapy. The following sections will focus on these aspects.

Aspirin as a prodrug

Prodrugs have been classically defined as derivatives of drug molecules which are bioreversible in nature and upon enzymatic transformation *in vivo* are converted into the active parent drug capable of showcasing the required pharmacological effect [79]. During the process of drug discovery there is significant emphasis on methods which minimize the undesirable effects of the drug and ameliorate the therapeutic value. One of the motives of prodrug development is to trim down the side effects of drugs. We have discussed the essential role of aspirin as an anticancer agent. However, effective inhibition of COX-2 and NF-κB can be achieved at high dose of aspirin. Furthermore, long term usage of this drug has been proven to be detrimental. Gastrointestinal side effects are the most predominant consequences of its usage [80,81]. It has been found to be responsible for a broad range of toxic side effects in the gastrointestinal tract including ulceration, gastrointestinal bleeding as well as perforation [82]. Hence, the development of aspirin prodrugs is gaining attention since it may help neutralize the negative aspects of the drug, thereby enhancing the therapeutic efficacy. Recent reports showcase the effect of such aspirin prodrugs in inhibiting the cancer stem cells. Kastrati et al [83] have developed aspirin ester drugs which conceal the carboxylic acid and enable inclusion of auxiliary pharmacophores in order to minimize toxicity and improve therapeutic effectiveness. The aspirin prodrug with fumarate termed GTCpFE displayed significant anti- NF-κB activity [84]. Alongside it also impeded mammosphere formation and expression of stemness marker CD44. In depth studies revealed that the fumarate moiety in the prodrug is predominantly responsible for the inhibition of NF-κB whereas both aspirin and fumarate moiety is required to showcase the anti-CSC effect. Additionally, the therapeutic effectiveness of diazeniumdiolate-based aspirin prodrugs against breast cancer was also evaluated [85]. The prodrug increased the level of oxidants which ultimately induced DNA damage followed by caspase-3 mediated apoptosis. Subsequently, non-tumorigenic cells remained unaffected by the aspirin prodrug. Apart from this, few reports also depict the usage of natural compounds for formulating aspirin prodrugs and have also evaluated their effectiveness as anticancer agents. Zhu et al [86] have synthesized novel resveratrol-based aspirin prodrug (RAH) which enabled release of aspirin along with resveratrol in order to diminish the toxic effects of aspirin. This prodrug impeded the expression of Cyclin D1 and Cyclin E and further induced apoptosis via the activation of caspase-3. In a similar finding, [6]-gingerol, which is the active compound of ginger, was used to synthesize a novel prodrug termed [6]-gingerol aspirinate (GAS) [87]. GAS reduced the expression of both COX-1 and COX-2 notably and hence improved

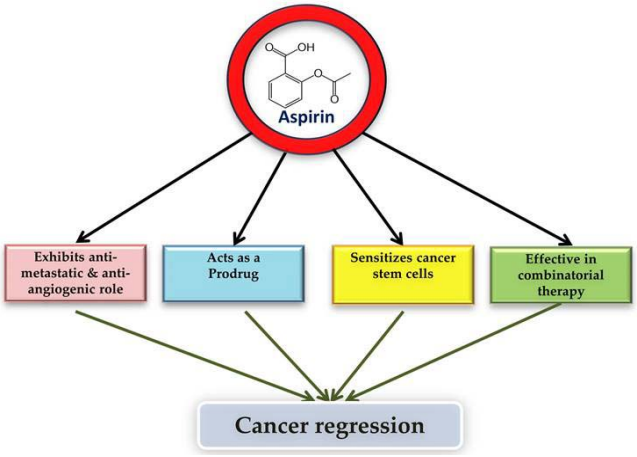

Figure 1: Repurposing of aspirin: The figure summarizes the lesser known therapeutic effects of aspirin apart from its conventional usage. It can potentially target the cancer stem cells which are the 'root' cause of cancer.

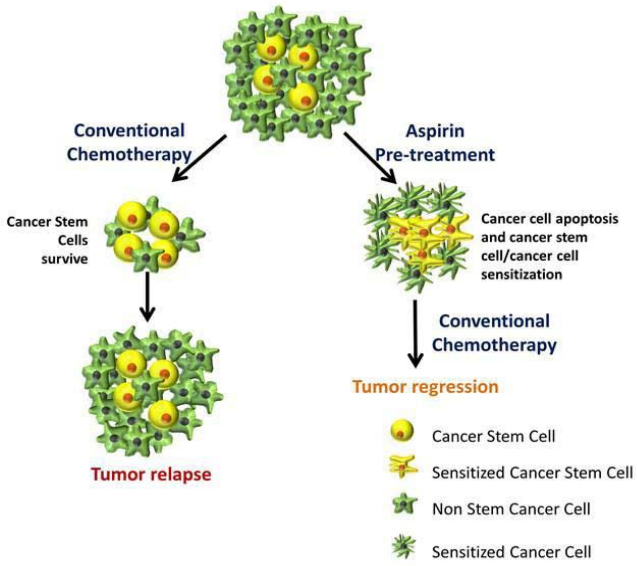

Figure 2: Effect of combinatorial therapy using aspirin: The figure displays the effectiveness of using aspirin in combinatorial therapy, where the resilient population of cancer stem cells is targeted. Conventional chemotherapy leads to tumor relapse. However, pre-treatment with aspirin negates this effect.

the anticancer effect of aspirin. The synthesized prodrug exerted gastroprotective effects in mice thereby nullifying the negative effect of aspirin. Therefore, prodrug formulations of aspirin are gaining popularity as they help enhance the pharmacological effects as well as diminish the harmful consequences of regular use. Furthermore, they may prove to be more efficient in targeting the CSC attributes along with negating the side effects.

Aspirin in combinatorial therapy

Therapeutic procedures involving single drugs often fail to eliminate the resistant CSCs as a result of which cases of tumor relapse are rampant. Hence combinatorial therapy, a treatment strategy which involves administration of two or more therapeutic drugs, is forming the crux of cancer therapy. The amalgamation of therapeutic drugs enables a synergistic approach which efficiently aids the obliteration of CSC population when compared to mono-therapeutic approaches [88,89]. Therefore, such treatment regimens are gaining popularity as they may help overcome the problems of drug resistance. With the growing number of reports stating the potential of aspirin in targeting both cancer cell and CSCs, an essential question arises that whether it can be successfully utilized for combinatorial therapy in order to aid a better treatment modality. Thus, administration of aspirin for sensitizing the resistant CSCs along with conventional chemotherapeutic drugs is gaining importance. A report from our lab exhibited that aspirin treatment prior to chemotherapy led to the sensitization of breast CSCs. It was found that chemotherapeutic drugs induced an inflammatory environment wherein the non stem cancer cells were converted to CSCs via IL-6 signaling which ultimately resulted in enhanced drug resistance and migration potential of CSCs. However, when upon aspirin pre-treatment, the nuclear translocation of NFκB was interrupted and hence IL-6 mediated CSC generation was also abrogated. It subdued the attainment of chemoresistance and CSC induction by hampering the NFκB-IL6 feedback loop [43]. Similarly, when aspirin was combined with gemcitabine for the treatment of PDA, the therapeutic efficacy of the chemotherapeutic agent was aggravated. The combination therapy of aspirin and gemcitabine successfully eliminated the CSC attributes along with tumor growth and invasion [67]. In an interesting finding, Gao et al [90] studied the acquisition of aspirin resistance upon treatment via induction of MCL-1. However, the combination of aspirin with the chemotherapeutic drug sorafenib resulted in considerable inhibition of cancer cells as sorafenib inhibited MCL-1 expression. Furthermore, constructive results were obtained in experiments involving xenograft models where the combination of aspirin and sorafenib resulted in tumor growth inhibition when compared to mono-therapy. In another report, the treatment of aspirin was combined with radiation therapy in cervical cancer to induce apoptosis. Promising results were obtained where aspirin pre-treatment enhanced sensitivity towards radiation therapy [91]. In ovarian cancer, a derivative of aspirin encompassing a nitro group sensitized the cells towards cisplatin. The ability of nitro aspirin to deplete cellular thiol enabled these cells to repossess cisplatin sensitivity [92]. In a recent report, aspirin was used in combination with Transarterial Chemo Embolization (TACE) which is a treatment procedure for hepatocellular carcinoma. The combined treatment strategy increased the overall survival of patients [93]. Therefore, the above findings shed light on the usage of aspirin along with other potential chemotherapeutic agents.

The above mentioned sections essentially highlight the dynamic role portrayed by aspirin in targeting the CSCs. Aspirin attacks multiple pathways in cancer stem cells which downregulate the self-renewal potential, impede metastasis and ultimately sensitize the CSCs towards conventional chemotherapeutic agents. Likewise, the prodrugs of aspirin also exhibit CSC inhibitory functions but with an added benefit by negating the side effects of the drug (Figure 1). Thus, usage of aspirin to target the CSCs is emanating as a promising therapeutic strategy.

Conclusion

The unavoidable long time lag required in the synthesis and final

clearance of new drug molecules for the final release into the market, calls for some urgent alternatives. In the meantime, the concept of drug repurposing has gained steam. This process had been accepted by the pharmaceutical companies, as it provides a relatively cheaper and definitely faster alternative. In this process, for finding new functions of existing drugs, researchers were particularly fascinated with the anti-inflammatory drug aspirin. Here, we furnished overwhelming evidences to demonstrate the anti-cancer effect of aspirin. Apart from lowering the risk of cancer, aspirin induces apoptosis in cancer cells while inhibiting neo-angiogenesis and retarding metastasis. This FDA-approved drug even targets cancer stem cells, the 'culprit' behind initiation, development, progression and drug-resistance of cancer and thwarts the self-renewable property of the CSCs. The conventional chemotherapeutic regimens wipe out the bulk of the tumor but fail to eliminate the cancer stem cells, as a result of which cases of cancer relapse are rampant. Interestingly, usage of aspirin in combinatorial therapy in due course led to cancer regression. Pre-treatment with aspirin has been found to sensitize the cancer stem cells which then can be easily targeted by conventional chemotherapeutic drugs (Figure 2).Therefore, due to the plethora of usefulness, there are no reservations regarding why aspirin has been called a 'wonder drug'.

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