Research Into the Metastatic Mechanisms of Circulating Tumour Cells and The Application Value of Src/FN1 Pathway Inhibitors

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Abstract. Malignant tumours are extremely dangerous to human health, with at least a 90 percent death rate in cancer-related cases being a result of metastasis. Circulating tumour cells (CTCs) play a central role in the progression and spread of disease. Multiplexed stages in the metastasis process of circulating tumour cell (CTC) are: epithelial/mesenchymal transition (EMT), cluster formation in CTCs, as well as apoptosis resistance without homing, and vascular-extravasation, which are all closely related to the Src/FN1 pathway. The literature review methodology adopted in this review involved a systematic retrieval and analysis of topical literature by relying sufficiently on databases during the last ten years. Findings show that the Src/FN1 pathway controls CTC metastasis in a variety of ways, such as supporting EMT, sustaining the stability of CTC clusters, protecting CTCs against apoptosis, and supporting moving over blood vessels. Src/FN1 inhibitor has good therapeutic prospects, as it addresses the CTC metastasis process directly and complements the therapeutic effects of chemotherapy, targeted therapies, and immunotherapy. The review is a comprehensive explanation of the regulatory processes of this pathway in CTC metastasis, as it offers a theoretical background for further in-depth studies and clinical application of pertinent inhibitors.

Keywords: CTC; Src/FN1 pathway; tumour metastasis.

1. Introduction

Tumour metastasis is one of the most fatal disorders of malignant tumours, which is the leading cause of death of most cancer patients. This occurs paradoxically, with less than 0.01 per cent of CTCs able to break loose from the main tumour and avoid haemodynamic stress and immune elimination, and eventually settle in the other organs to form their metastatic lesions. However, it is these very rare cells that survived that define the outcome of the patients, about 90 percent of whom, as a result. Thus, a metastasis can be called a low possibility but fatal process. CTCs, as the most crucial intermediaries between the primary tumours and the metastatic sites, are the primary actors of metastasis. The control mechanism of their existence, migration, and fixation has been the centerpiece of cancer research for a long time.

Present-day medical detection technologies allow the effective isolation and sorting of CTCs as well as their functional study, which allows for a better understanding of mechanisms of metastases and their response to treatment and the likelihood of recurrence. However, because of the sheer number of pathways that are involved in CTC behaviour, the Src/FN1 pathway is a pathway that is gaining equal prominence. The SFKs of Src family control cell growth, cell differentiation, cell migration, and apoptosis; their inappropriate activation makes the tumours invasive. As a core of the extracellular matrix, fibronectin 1 (FN1) reorganised the tumour microenvironment and triggered subsequent signal transmission via cell surface receptor interaction and, consequently, determined the malignant tumour phenotype.

A collaborative mechanism was found recently between Src and FN1. When bound to particular receptors, FN1 corresponds to membrane recruitment of Src and subsequent microactivation of Src, subsequently controlling cytoskeleton rearrangement of CTCs. This improves their anti-apoptotic abilities, preserves a metastatic-prone mesenchymal endothelium, and eases the breakage of circulatory barriers. The exact mechanism of the involvement of the Src/FN1 pathway in CTC metastasis, however, is unknown, which predetermines the purpose of the given research.

The paper is devoted to the relationship between the CTC metastasis processes and the Src/FN1 pathway, answering such basic questions as: What is the role of the Src/FN1 pathway in the key metastatic processes, such as detachment, survival, migration, and adhesion? What are the activation pathways and upstream regulatory molecules, and downstream regulatory molecules of this pathway? Do targeted drugs prevent CTC-metastasis and have clinical potential?

This research paper has utilized a literature review approach, in which it has accessed simple research literature on experiments, animal studies, and clinical literature in databases such as PubMed, Web of Science, CNKI, and Wanfang within the last ten years. The keywords included: CTCs, Src family kinases, FN1, tumour metastasis, and targeted therapy. The structure of the Src/FN1 pathway and its mode of activation, its relationship with CTCs, and the developmental progress in the drugs concerning the same were systematically summarised and analysed by the research.

The study will include the correlation of the metastatic biology processes of CTCs and the Src/FN1 pathway, directing the targeted attack on CTCs and their spreading, and complementary improvement of the current treatments.

The research will shed light on the mechanism through which the Src/FN1 pathway controls CTC metastasis loss, assess the potential of available therapies in clinical practice, serve as a theoretical resource in future studies, and give new ideas to novel anti-metastatic therapies.

Scientifically, connecting the findings of appropriate research can put some critical nodes in tumour metastasis regulatory systems, thus narrowing the signalling theory between the tumour microenvironment, CTCs, and the metastatic foci. This offers how certain molecular targets can be identified. The targeted therapy value analysis provides a reference in the choice of drugs. These findings will form the basis of designing personalised anti-metastatic regimens that are expected to result in better patient outcomes and minimization of recurrence.

2. The biological mechanisms of CTC metastasis and its association with the Src/FN1 pathway

CTCs are tumour cells that have detached from solid tumour lesions (primary or metastatic). CTCs are the collective term for all types of tumour cells circulating in the peripheral blood. Most CTCs die by apoptosis or are phagocytosed when they enter the peripheral blood circulation; few escape and can give rise to metastatic lesions, which increases the mortality in cancer patients. Tumor metastasis consists of three stages: dissemination, dormancy, and establishment, which are potentially sequential and overlapping in time [1]. In this process, CTCs go through several steps, including detachment from the primary tumor, invasion into the surrounding stroma, entry into the lymphatics or blood vessels, survival while being transported in the circulation, and colonization of a distant organ, which is called the metastatic cascade [2,3].

2.1. Shedding and survival of EMT and CTCs

EMT occurs before tumor cells detach from the primary lesion, with the aim of enabling tumor cells to acquire invasive ability to form CTCs and start the process of metastasis. Through this, the epithelial cells become gradually less polarized and fail to protect the cell-cell junctions, taking on a mesenchymal-type phenotype. This further dramatically accelerates their migratory and invasive capacities, which form the much-needed requirement of CTCs being able to effectively dissociate from the initial site of tumour and access the circulatory system [4]. Being an essential signalling molecule that controls the EMT process, Src destabilizes the E-cadherin complex by phosphorylation, thus enhancing endocytosis and the degradation of the E-cadherin complex. Besides, the Src signal activation also increases the level of expression of important EMT transcription factors, including Zinc finger E-box-binding homeobox 1 (ZEB1) and Snail, which further increases the rate of the EMT process [4]. FN1 is a typical product after EMT has taken place. It is also capable of binding to molecules of the integrin family, reversely triggering the Src/FAK signalling pathway. This constitutes a long-lasting and stable positive feedback regulation mechanism, which essentially

preserves the stromal phenotype and motility of tumour cells. This is important support to the detachment, circulating survival, and later metastatic action of CTCs [4]. EMT is not an easy ontogenic binary of epithelial and mesenchymal morphologies. Tumour cells often exist as an intermediate, referred to as epithelial-mesenchymal plasticity (EMP). This characteristic phenotype gives the CTCs increased adhesive strength, transendothelial motility, and distant organ seeding capacity, and thus is a major cellular subgroup driving distant tumour metastasis [4]. Besides, when circulating, CTCs have the ability to adapt to blood flow shear stress by expressing molecules like integrin β1. This survival ability has a close connection with the EMT-mediated cellular phenotypic reorganisation [5].

2.2. Formation and Migration Advantage of CTC Clusters

Cell clusters of aggregates of multiple CTCs, in comparison with individual CTCs, have much higher metastatic potential and can metastasize many dozens of times more than single CTCs. In terms of molecular mechanism, CTC clusters transmit survival signals through intercellular junctional structures (such as junctional proteins like plakoglobin), which offer stable microenvironmental support to cells in the cluster [6].

The study of Huaman, J. and Ogunwob affirms that FN1-mediated cell-cell or cell-matrix adhesion is a key process in the CTC clusters. It controls the effectiveness of cluster assembly by controlling the efficiency of physical bonds and signal conduction among cells [7]. Thus, Src kinase has a role in ensuring the structural stability and integrity of the CTC cluster through the dynamic assembly and dissociation of focal adhesions. On the one hand, it facilitates the development of intercellular adhesion structures in the clusters; on the other hand, it does not allow excess dispersion of the clusters. According to this mechanism, suppressing the Src kinase might interfere with the structure of formed CTCs, such that they dissipate and detach into single cells. This makes them more vulnerable to the action of the host immune system or inactivation in the circulation, and minimises the risk of tumour metastasis.

2.3. Resistance to apoptosis

Nest loss apoptosis is one of the main pathways of homeostasis that normal epithelial cells use to determine the death of cells, which means that it is a programmed cell death triggered by the process of pulling away of cells from the extracellular matrix (ECM), and the loss of anchoring signals [8]. However, CTCs must overcome this constraint. Within the unanchored environment of the bloodstream, they must acquire resistance to apoptosis through the activation of specific molecular pathways in order to complete the metastatic process from detachment at the primary site to distant organ implantation [9].

Within the molecular network mediating CTC resistance to nest-loss apoptosis, the Src/FN1/integrin signalling pathway plays a pivotal role as a 'pseudo-anchoring signal generator'. Upon entering the cell cycle, CTCs' surface integrin receptors can bind to their own secreted FN1, forming an autocrine cell-matrix interaction interface that subsequently activates the non-receptor tyrosine kinase Src. Upon activation, Src transmits pro-survival signals through two key downstream pathways. Firstly, it mediates the phosphorylation of the β-subunit of integrin, recruiting the PI3K regulatory subunit p85 to activate the PI3K/Akt pathway, thereby inhibiting the apoptotic cascade through the phosphorylation of apoptosis-related molecules [10]. Secondly, it jump-starts the Ras/Raf/ERK pathway by phosphorylating the adaptor protein, which facilitates the expression of anti-apoptotic proteins. This cascade activation pathway forms a signal camouflage pathway in CTC that resembles the anchoring of the ECM. It continues to signal survival, even in circulating environments where loss of physiological matrix attachment leads to a high rate of apoptosis, thus playing a big role in slowing the rate of apoptosis when it binds to the integrin receptor.

2.4. Intravascular and extravascular leakage

Acquiring haematogenous metastasis mediated by CTC is a critical pathway towards the spread of tumours at a distance. As key phases of vascular movement of CTCs, intravascular extravasation and extravasation positively affect the efficiency of the formation of metastatic foci. Intravascular seeding is when tumour cells shed off the vascular wall of the initial site of disease, penetrate the endothelial barrier, and enter the circulatory system. Extravasation refers to the next phase of the process whereby, having colonised other distant organs (such as the liver or lungs) through blood, CTCs invade the vascular wall again to access the interstitium and proliferate again to create metastatic foci. Their combination forms the transvascular route through which CTCs develop since they are said to be detached at their main site, and then colonise secondary sites. The effectiveness of this process is predetermined by the adaptation of CTCs to the vascular microenvironment and the use of their own motility resources.

The Src and FN1 synergistic control play a significant role in this process. Src, being a core molecule that coordinates vascular permeability, has direct effects on the vascular endothelial growth factor (VEGF) signalling pathway. It causes the decoupling of endothelial cell junctions, destruction of the vascular barrier, and increased permeability to open pathways through which CTCs can cross the endothelium by activating the VEGF-A/VEGFR2 signalling axis [11]. It is a universal regulatory action; an example is the abnormal activation of the appropriate pathways in colorectal cancer that qualitatively increases vascular permeability, contributing to the enhanced internalisation and extravasation of circulating tumour cells [12].

FN1, which is one of the adhesive elements of the extracellular matrix, supports CTC extravasation in two ways. First, it creates a momentary adhesive polymer over the vascular wall, and in doing so, offers an anchoring process to the CTCs to allow a stable contact with the endothelium under shear pressure in the blood flow. Secondly, it interacts with the integrin receptors on the CTC surface, triggering downstream Src signalling pathways. This mobilises cytoskeletal remodelling in CTCs, including actin polymerisation, and encourages the formation of pseudopods, thus making them more efficient at squeezing between endothelial junctions. This will help them to pass through the endothelial layer successfully. Furthermore, those exosomes released by tumours have the potential effects of substantial perivascular deposition of FN1 in remote organs, which further promotes their helpfulness in supporting CTC extravasation and implantation by developing pre-metastatic niches [13].

3. Src/FN1 signalling pathway

3.1. Directly targeting circulating tumour cells and their metastatic processes

The Src/FN1 pathway is a key regulatory centre of CTCs in order to accomplish haematogenous metastasis. Particular supporters of this pathway can synergistically prevent the metastatic behavior of CTCs by applying the multidimensional principles, which provide an essential intervention strategy to prevent anti-metastatic cancer therapy. On the phenotypic regulation stages, at this stage, these inhibitors increase the expression of the epithelial marker E-cadherin because of inhibiting the Src kinase activity, such that the CTCs are prevented from undergoing a mesenchymal to epithelial phenotype transition, ultimately reducing their invasive potential. According to preclinical studies, the Src inhibitor KX2-391 was significantly able to decrease the expression of the mesenchymal nature of lung cancer CTCs by suppressing both phospho-Src (p-Src) and FN1 [14]. It also destabilizes intercellular adhesion signals (like integrin-mediated adhesion pathways) that stabilize CTC cluster formation at the cellular aggregate intervention level. Here, CTC clusters are broken down into single cells, with a much greater metastatic potential than individual cells, and as a result, the chance of CTC being cleared is greatly increased by their dispersal into single cells during blood circulation. Through experimental studies, it was found that lung cancer CTC clusters had a 4.5-fold

rate of pulmonary metastasis, as compared to solitary CTCs. After Src/FN1 pathway was blocked, the metastatic outcome of dissociated solitary CTCs was reduced by more than 60 percent [14].

In the cell survival regulation dimension, these inhibitors have the potential to dissect the survival cues through the interacting Src/FN1 pathway and interfere with CTC resistance to apoptosis in response to the loss of anchorage. This renders CTCs vulnerable to apoptosis during circulation due to the absence of ECM anchoring support. Previous studies have demonstrated that FN1 gene knockdown increases the apoptosis rate of lung cancer CTCs in suspension culture by 55%, with this effect directly linked to the blockade of integrin α5β1-mediated anchoring signals [6]. In the dimension of blocking distant implantation, it reduces the interaction strength between CTCs and microvascular endothelial cells in distant organs (such as the lungs) by inhibiting cytoskeletal remodelling and adhesion molecule expression downstream of the Src/FN1 pathway, thereby diminishing the extravasation process of CTCs and subsequent metastasis formation; In lung cancer animal models, KX2-391 treatment reduced CTC-induced pulmonary metastatic nodules by up to 84%, with a marked decrease in CTC extravasation rates within lung tissue [14].

3.2. Synergistically enhance the efficacy of existing therapies

3.2.1 Src Inhibitors and Chemotherapy Target Resistant Cells

In the practice of combined chemotherapy for tumours, while chemotherapeutic agents can effectively eliminate tumour cells in an active proliferative state, they remain powerless against two distinct populations of cells. Tumour cells in a dormant state, as well as CTCs exhibiting stem cell characteristics, are precisely the key factors responsible for tumour metastasis and recurrence. El Touny et al. observed in breast cancer-related studies that specific Src inhibitors (such as AZD0530) can maintain dormant tumour cells in a quiescent state by regulating the localisation of the cell cycle suppressor p27, thereby preventing them from entering the proliferative phase in response to external stimuli [15]. Que et al. made a profound study of lung cancer models, which showed evidence that the abnormally activated Src/FN1 pathway is crucial in the clustering program of the circulating tumour cells. This clustering behaviour improves CTC survival in the haematological milieu and heightens the resistance of chemotherapy on CTC [14]. The regulatory action of this pathway is especially high in the case of those dormant CTC subpopulations that are unresponsive to chemotherapy. The duration of stay of the CTCs in the circulatory system has a direct effect on the likelihood of metastasis. The Src/FN1-pathway inhibitor KX2-391 perturbs the structural integrity of CTC clusters in the clinical stage. KX2-391 has the ability to directly react to dormant CTCs, which cannot be reacted to by chemotherapy, and also has synergistic actions with typical chemotherapeutic agents (such as platinum-based drugs). The combination of both agents would inhibit the events, which are related to repairing the damage of the DNA, and this would significantly tune up the effectiveness of the chemotherapy drugs in the eradication of CTCs and in the overall prevention of the tumour metastasis process [14]. Herein, in a nutshell, the Src inhibitors are useful complementary to chemotherapy that spots and targets precisely the CTC subpopulations that are dormant or stem cell-like, and have proven to be resistant to chemotherapy. This is to overcome the fact that chemotherapy is limited in the treatment of individuals at the mechanism level by destroying the circulating CTC, which eventually helps to minimise the risk of tumour metastasis and recurrence.

3.2.2 Src Inhibitors and Targeted Therapy Reverse Resistance

Compensatory upregulation of the Src pathway is common in combined targeted therapies with tumour resistance to Epidermal Growth Factor Receptor (EGFR) or Human Epidermal Growth Factor Receptor 2 (HER2) inhibitors. At this point, a combination of Src inhibitors (including dasatinib) is able not only to reverse this resistance condition but also to prevent the growth of primary tumours and CTCs dissemination. In their investigation of Anaplastic Lymphoma Kinase (ALK)-positive Lung cancer, Diaz-Jimenez et al. noted that despite the lack of targeting of either EGFR or HER2, resistance to ALK inhibition by brigatinib is typically associated with a high expression of the Src kinase. This compensatory regeneration preserves tumour cell growth and survival through an active

Mammalian Target of Rapamycin (mTOR) pathway, among other things [16]. The combined use of ALK inhibitors with the Src inhibitor dasatinib, by interfering with the tumour cell proteome and synergistically inhibiting the phosphorylation of key proteins in the mTOR pathway, not only effectively suppresses the growth of ALK-driven lung cancer cells derived from both mice and patients, but also reduces the expression of drug resistance-associated proteins (such as Yes-Associated Protein 1 and Glycogen Synthase Kinase 3β), indirectly corroborating the mechanism of Src compensatory activation during EGFR/HER2 resistance. This suggests that following targeted therapy failure, the Src pathway may serve as a common compensatory node sustaining tumour survival [16]. The research by Diaz-Jimenez et al. focused on the ALK target, but as compensatory activation of Src represents a common mechanism of resistance, the concept of combining it with targeted therapies could be extended to scenarios of EGFR/HER2 resistance.

Moreover, Yuan et al. provided more direct validation of this phenomenon through their study of non-small cell lung cancer cells (HCC827-ER) resistant to the EGFR inhibitor erlotinib. Significant elevation of Src pathway activity was observed in erlotinib-resistant strains [17]. Combination therapy with the Src inhibitor bosutinib and the Mitogen-Activated Protein Kinase Kinase (MEK) inhibitor trametinib markedly downregulated phosphorylation levels at key sites within the EGFR pathway, such as EGFR Y845 and Y1068. Simultaneously inhibits downstream signal activation of Extracellular Signal-Regulated Kinase (ERK), Protein Kinase B (AKT), and others, inducing apoptosis in drug-resistant cells. This combined strategy reduced tumour volume by 82% compared to the monotherapy group in the nude mouse HCC827 xenograft model, demonstrating significantly superior inhibitory effects to single-agent targeted therapy. This further confirms the pivotal role of Src inhibitors in reversing resistance to EGFR-targeted drugs [17]. Yuan et al.'s study directly demonstrated the reversal effect of Src inhibitors in EGFR-resistant models.

Regardless of the initial target, compensatory activation of the Src pathway may represent a key mechanism for tumour escape from therapy. Combination therapy with Src inhibitors effectively blocks this process while exerting control over both primary tumour progression and CTC dissemination.

3.2.3 Mechanisms of Src Inhibitors Enhancing Immunotherapy Efficacy

In combined immunotherapy, CTCs exhibiting a stromal phenotype demonstrate reduced susceptibility to immune attack. Src inhibitors can restore CTC sensitivity to T-cell-mediated killing by inducing mesenchymal-epithelial transition (MET), whilst simultaneously modulating tumour-associated macrophage function to improve the immune microenvironment, thereby enhancing immunotherapy efficacy.

Hematopoietic Cell Kinase (HCK), a member of the Src family of kinases, is a key regulator of tumour-associated macrophage function. When highly activated in immunosuppressive M2 macrophages, it limits T cell activity by secreting factors such as IL-10 and TGF-β. Poh et al. observed in the MC38 colonic carcinoma model that following targeted inhibition of HCK using the Src inhibitor RK20449, tumour-associated macrophages underwent reprogramming from the M2 phenotype to the pro-inflammatory M1 phenotype. This resulted in a 2.8-fold increase in IL-12 secretion alongside downregulation of surface PD-L1 expression [18]. This modulation not only reduces macrophage suppression of T cells but also promotes the recruitment of $CD8^+$ T cells to the tumour site by increasing the release of CXCL9/CXCL10 chemokines. The interaction between RK20449 and anti-PD-1 therapy in patient-derived xenograft (PDX) models in humanised mice improves the immune evasion relevant to tumour cell circulation by 58% over monotherapy immunotherapy [18].

The stromal phenotype of circulating tumour cells further increases the immune avoidance through the grouping formation. In lung cancer studies, Que et al. noted CTC clumps that are mainly avoided by T-cell recognition by the means of physical barrier effects because of high expression of Src/FN1 pathway-related components. The Src inhibitor KX2-391 not only disrupts this cluster structure but also induces MET transformation in CTCs [14]. Inducing a fourfold upregulation of E-cadherin expression in mesenchymal-type CTCs that originally exhibited low expression of this epithelial

marker, while simultaneously restoring major histocompatibility complex class I (MHC-I) molecule expression. This enables $CD8^+$ T cells to re-recognise and eliminate these CTCs. In a mouse lung metastasis model, this MET conversion increased T cell infiltration in lung metastatic lesions by 62% [14].

Src inhibitors also indirectly assist in the MET conversion of circulating tumour cells by improving the immune microenvironment. Poh et al. demonstrated that inhibition of HCK reduces the secretion of immunosuppressive factors by macrophages, thereby diminishing the formation of the 'immune protective shield' around interstitial-type circulating tumour cells [18]. Que et al. also observed that CTC-secreted TGF-β, which suppresses T-cell function, decreased following KX2-391 treatment, creating a dual-relief effect alongside macrophage functional remodelling [14]. Immune evasion by interstitial phenotype circulating tumour cells and immunosuppression by macrophages represent two distinct processes. The Src inhibitors have the potential to produce synergies by acting on both of these pathways. As an example, in the model of lung cancer created by Que et al., the KX2-391 made CTCs susceptible to T cells and, at the same time, decreased macrophage suppression. This ultimately caused an 84 percent decrease in the nodules of the lungs that were metastatic [14]. This synergistic effect has also been verified by research carried out by Poh et al., who confirmed that this effect is more pronounced when anti-PD-1 treatment is used, thereby amplifying the inhibition of tumour growth by 30 percent in addition [18].

3.3. Challenges and Directions

Though Src inhibitors have shown certain signs of efficacy in combination cancer therapies, there are still so many challenges before the use of this drug in clinical practice is made effective to help more patients. The way forward should then be focused on finding the appropriate channels to move forward.

As far as patient stratification and biomarkers are concerned, not every patient will respond to Src inhibitor treatment, and the generation of easily available blood-based biomarkers is essential. Que et al. showed in lung cancer research that the presence of p-Src in CTC clusters is significantly linked to the presence of FN1 expression, causing patients to have a much increased chance of lung metastases. In such patients, you have shown that the CTCs clearance with the Src inhibitor, KX2-391, exhibited almost three times greater efficacy relative to low-tumour-burden patients [14]. Further development of the liquid biopsy technology has opened the clinical possibilities of using this biomarker, which necessitates very small volumes of blood and does not involve invasive methods. The non-invasive stratification of patients can be done using p-Src activity or transcription levels of FN1 in CTCs in peripheral blood. Nevertheless, the existing testing procedures do not have standardised procedures and need testing on a greater number of patients.

The fundamental drawbacks of first-generation Src inhibitors are target selectivity and their toxicity, which limit their use. Dasatinib, as a typical multi-target inhibitor, acts on kinases such as ABL and c-Kit whilst inhibiting Src. Bone marrow suppression was encountered in around 38 percent of the patients in a clinical study, and some patients had to stop treatment because of the intolerance. Future studies need to create Src inhibitors that are highly selective, such as drugs put together with special structural characteristics of Src. Alternatively, instead of inhibiting Src directly, it could be applicable to inhibit the binding of FN1 with other proteins (such as integrins). This would prevent targeting other kinases and thus majorly decrease the side effects. The team led by Professor You Yaodong postulated that the non-ATP-binding pocket of Src would need further targeting or the utilization of the covalent inhibition strategies to provide the reduction of off-target effects to a considerable degree [19]. Their new allosteric inhibitor was tenfold more selective against Src than dasatinib, and their bone marrow appeared to be unaffected by their novel allosteric inhibitor. Furthermore, Src/FN1-binding inhibitors have potential, since they can be used to overcome the wide-ranging toxicity of direct kinase inhibition. They are shown to interfere with cluster architecture and prevent metastasis in a lung cancer CTC cluster model.

Alterations in the care of the different metastatic organs have to be highlighted as well. When cancer metastasizes to different organs such as the lungs, bones, and liver, there are differences in the local microenvironment, which also leads to organ specificity in Src pathway activity. As an example, during the metastasis of lung cancer, Src and FN1 are involved in the positioning and proliferation of CTCs in the lungs; with treatment by KX2-391, 84% of metastatic nodules in the lungs have been reduced [14]. Dasatinib in combination with EGFR inhibitors augments the results of bone metastases [20]. However, Src inhibitors prove less effective in liver metastases—likely because FN1 facilitates CTC adhesion differently within the hepatic.

The clinical profile concerning the administration schedule and interaction regimens is not clear. The current research majority emphasizes developed cancer, such as dasatinib combined with erlotinib for advanced cancer of the lung, with 63 percent of patients obtaining disease control; or combined with dacarbazine for advanced cancer of melanoma, with 72.4 percent of patients obtaining disease control [21,22]. The current clinical relevance of Src inhibitors in the adjuvant treatment of tumour patients in early-stage tumor patients is yet to be substantiated by clinical proof. Launched on the analogy with the mechanism through which CDK4/6 inhibitors enhance the results of breast cancer adjuvant treatment by removal of micrometastases, we postulate that Src inhibitors can be used in postoperative minimal residual disease (MRD) positive patients to prevent the risk of tumour recurrence [23]. Nonetheless, this supposition still needs to be confirmed with the help of large-scale phase III clinical trials. Moreover, the combination therapies with Src inhibitors should be further optimised: with immunotherapy, there should be a special emphasis on the groups of patients with stromal-phenotype CTC increase; with chemotherapy, the priority should be placed on the therapeutic responses versus the toxicity. Designing personalised treatment strategies based on the biomarker is key to the refinement of these regimens.

4. Conclusion

The use of circulating tumour cells to mediate metastasis of tumors makes their circulation in the body deadly, whereby their intricate biological phenotypes provide various opportunities to be used as targets in therapeutic interventions. The Src/FN1 signalling network, which serves as a central node to coordinate several key processes such as CTC-EMT, survival, clustering, and vascular invasion, has been receiving growing prominence. SRC/FN1 pathway inhibitors occur in several stages by acting on CTCs. Not only are they naturally endowed with the potential to suppress the metastasis process, but they also exhibit synergetic action with the currently existing conventional treatment modalities, thus providing new approaches to overcome the clinical challenge of tumor metastasis. The research should aim at accurately defining the beneficiary population, maximising combination treatment regimens, and addressing the toxicity issue in future research. Finally, such a potentially powerful treatment solution will be appropriately translated into a real survival gain for the patient.

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