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Review

Single Cell Editing: Applications of Multi-omics Profiling of Single Cells

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Abstract. The highly integrated data obtained from diverse omics fields has enabled us to study complex relationships among organisms and to identify novel genotypic and phenotypic targets of lethal diseases. In the age of precision medicine, data obtained from various omics modalities such as transcriptome, epigenome and metabolome prove to be very useful in health and medicine. The data combined from different omics levels also proved to be very fruitful in the study of different types of cancer at the single cell level. In addition to cancer, a single cell study provides deep insight into the underlying mechanisms of various infectious diseases. Besides this, single cell technology is also helpful in discovering new cells and in tracing ancestral lineages of different species. Moreover, the role of our immune cells in response to these infective agents and new drug targets can also be studied using these omics technologies. These different omics levels provide authentic combined data and hence they are helpful in stem cell research. Circulating tumor cells, prenatal diagnosis of fatal diseases and lung malfunctioning can only be studied at single cell resolution. This review article aims to comprehend the applications of omics technology in the study of lethal diseases which would only be possible at the single cell level and to find potent drug targets.

Keywords: single cell multi-omics, profiling, cancer cells, noval genotype

Introduction

All living things ranging from simple unicellular organisms to complex eukaryotes are composed of a **c**ommunity of individual cells. The cell is the basic fundamental unit of a living system and the study of different organs at a single cell level is critical to understand their diverse biological complexity (Mincarelli *et al*., 2018). Multi-omics approaches are gaining wide acceptance in the field of health and medicine because they allow the integration of data from multiple omics platforms. Single cell sequencing technology deepens our understanding of phenotypic and genotypic variations in bulk tissues because these approaches provide a valuable opportunity to measure the extent of different molecules such as DNA, RNA and proteins with high resolution. For example, genomic DNA and RNA from a single cell can be used to assay single cell genome, proteome and transcriptome respectively. These single cell omics technologies help to develop a multi-omics profile of the same cell (Hu *et al*., 2018). Multi-omics is a multifaceted approach and it consists of two main steps. The first step is a random selection of single intact viable cells from a heterogenic population. The second step is the enzymatic dissociation of these single cells by micro pipetting, serial dilution and microfluidic platforms (Wang and Navin, 2015).

In the era of precision medicine, single cell omics technologies help to determine the interaction of tumour cells in their local micro-environment. These interactions can be used to identify biomarkers for cancer diagnosis, prognosis and new drug targets. With the aid of these single cell methods the human tumor atlas network (HTAN) framework has been developed which relies on mapping tumour atlases at molecular, cellular and clinical levels and aims to interrogate single cell data thoroughly for clinical transitions (Liu *et al*., 2021). The single cell omics approaches are also helpful in studying complex mutational mechanisms and the complex structure of tumour mass (Helleday *et al*., 2014). Thus, the study of cancer cells at an individual level will un-doubtedly enhance our understanding of all facets of tumour biology (Baslan and Hicks, 2017).

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Single cell sequencing methods are employed study for cancer stem cells. The stem cells are important biomarkers to study the mutations which can disrupt normal cellular pathways such as apoptosis. Moreover, they have the potential for self-renewal and they differentiate into specialized cells (Chen *et al*., 2020). Single cell omics methods have brought a revolution to the study of different types of cancer particularly breast cancer. Breast cancer is known for its distinct clinical features and patient outcome despite having the same his-topathological features. Classical therapeutics rely on the classification of tumours based on estrogen or progesterone receptors expression but this classification is only fruitful for a limited time. Single cell sequencing methods proved to be very effective in this manner as they overshadow the problem of heterogeneity and also predict the long-term survival of targeted therapies (Dwivedi *et al*., 2019).

Single cells sequencing methods are also used to treat lung adenocarcinoma and neurological disorders. These methods are very helpful in finding the causative agents which can cause drug resistance to novel drugs (Peng *et al*., 2020). The nervous system consists of billion interconnected neurons which are diverse in their morphology and function. The genomic composition of these nerve cells also differs from each other. Single cell sequencing methods are helpful in the classification of these nerve cells (Li *et al*., 2017). It is a fact that tumour cell heterogeneity is very significant during the development of the initial nephron at an embryonic stage. Single cell sequencing methods are used to study this molecular heterogeneity during critical stages of the development of human renal cells. These techniques are helpful to study gene expression profiles of the renal cells along with crucial transcriptional regulatory elements (Wang *et al*., 2018).

Recent advancements in microfluidics technologies such as nano-well arrays, flow cytometry and single cell multi-omics profiling show promising results to develop therapeutics and study immune responses at single cell level (Choi, 2020). Single cell sequencing techniques mostly rely on monitoring host immune responses against different diseases as these responses differ among individuals, molecular mechanisms of the diseases and then finding novel drugs to treat these diseases (Tian *et al*., 2022).

Tumour cells show heterogeneity due to which they are difficult to treat. Single cell omics methods help study and analyze circulating tumor cells (CTCs) which provide a deep insight into the tumor initiation, progression and metastasis process. These biomarkers are efficient tools used for treating multiple cancers and developing novel drug targets (Alix-Panabières and Pantel, 2013). The complexity in the treatment of breast cancer patients arises due to the production of disseminated tumour cells (DTCs) from primary carcinoma. These DTCs are released into bone marrow and result in distant metastasis. Single cell sequencing methods are helpful in the detection of these circulating and disseminated tumour cells which ultimately increases the chances of patient survival (Riethdorf and Pantel, 2008).

There are many fatal diseases which if detected and treated at the embryonic stage can result in healthy birth. Single cell sequencing methods are used in the prenatal diagnosis of lethal diseases such as SNPs (Pan, 2014). Single cell-omics techniques are also helpful in studying various pulmonary diseases such as asthma and chronic obstructive pulmonary disease (COPD) Fig. 1, reported by (Hardin and Silverman, 2014).

Single cell omics studies help us to understand the pathological pathways involved in disease initiation and progression, thus leading to the development of effective therapeutic measures. As single cell sequencing was successfully applied to several neurological disorders and in cancer research, this review article aims to develop an understanding related to single cell omics methods and their role in different diseases like cancer, pulmonary disorders and infectious diseases which would only be possible at the single cell level and to find potent drug targets.

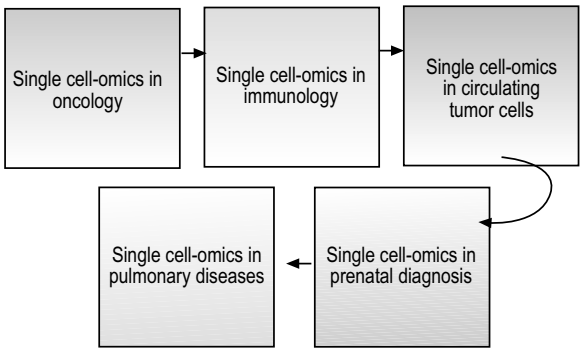

Fig. 1. Single cell omics, a multifaceted approach to studying lethal diseases.

Omics technology enabled different modalities of single cells such as transcriptomes, proteomes and genomes. Previously, most review articles focus on different omics techniques and their applications. The current review article particularly focuses on the applications of single cell omics techniques in the study of different types of cancers, lung diseases, infectious diseases and prenatal diagnosis, thus helpful for researchers to investigate the complex pathological pathways involved in different diseases (Fig. 2).

Single cell sequencing applications in the diagnosis and therapeutics of disease. ***Single cell omics oncology.*** Recent advancements in genomic technology have permitted the measurement of mutations and faithful detection of mutations at the single cell level. Moreover, gene expression profiles of cancer cells can also be studied. The human body is made up of approximately forty trillion cells (Zhang *et al*., 2016). Various exogenous and endogenous signals which mediate specific RNAs and proteins control cellular diversity.

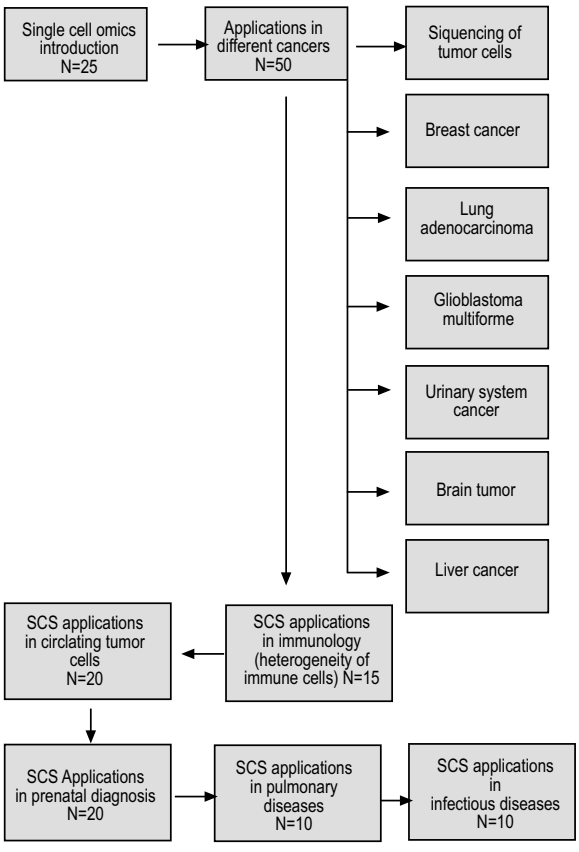

Fig. 2. Study flow diagram.

However, it was traditionally thought that DNA is a stable molecule and comprises individual genomes (Paulsen *et al*., 2009). The most common example of genomic mosaicism is cancer mosaicism. In this specific kind of mosaicism cancer, cells undergo genetic changes during the formation of tumour cells. Most carcinomas exhibit polyclonal nature which further complicates genomic heterogeneity with populations of tumor cells. These alterations in the genome are so prominent that they are not only distinct from the host genome but also from other nearby cells. The survival rate of patients declined due to these reasons and the treatment procedures are also affected. Drug-resistant populations of cancer cells may develop if diagnosis primarily consists of a single biopsy because it shadows the heterogeneous nature tumor. This ultimately leads to the false detection of all active variants (Gajecka, 2011). Designing such kinds of drugs that are used to treat primary tumors may not be effective to treat metastases. Because they may be originated from minor sub-clones within the primary tumor or it might be possible that they have acquired new mutations. Therefore, for effective patient care, it is important to undergo thorough screening of genome heterogeneity in cancer at the single cell level (Navin *et al*., 2011).

Recently, various methods are being developed to study cancer genetics precisely. These methods have been an effective tool to study cancer cells effectively because cancer cells exhibit high heterogeneity and abrupt transformations in populations of cancer cells, mutations, and various complex procedures involving clonal evolution mechanisms. These methods also enable the identification of novel biomarkers of variant tumors. The knowledge obtained from these methods is very fruitful as it helps in studying the early detection of rare cancer cells, such as CTCs and DTCs and they promote the concept of personalized medicine (Chung *et al*., 2017 .

Single cell sequencing of tumor cells. ***Cancer stem cells*****.** Normal stem cells are quite rare and they can remain undifferentiated for a longer period. They generate morphologically distinct progeny cells due to their self renewal ability (Boman and Wicha, 2008). Different types of tissues are particularly specific to certain tissues and thus are important for growth and development because they can regenerate themselves. Cancer stem cells may be developed from tissue specific stem cells due to their ability to accumulate mutations over time that initiate carcinogenesis. These mutations

along with other additional mutations in cancer stem cells take place during tumorigenesis. They may have serious repercussions as they can disrupt normal molecular pathways such as apoptosis, normal growth processes and differentiation. This in turn contributes to genetic diversity in clonal cells within a primary carcinoma (Dontu *et al*., 2003). Nowadays, single cell sequencing may be used to study cancer stem cells to study the key mutations that may disrupt the functional pathways which promote the process of tumor production. In many aspects of cancer biology stem cells are key players in various processes such as tumorigenesis, metastasis and drug resistance so the primary goal of modern anti-cancer therapeutics is the eradication of these stem cells. Stem cells are a useful tool to study functional heterogeneity at a single cell level but their practical utilization is very limited. Because RNA is present in a very small amount in a cell hence this method cannot be used on a large scale (Wicha *et al*., 2006).

Single cell gene expression profiling has been proved by a handful of techniques in several studies. It has been used to identify important regulatory networks such as EMT in the study of stem cells (Lawson *et al*., 2018; Akrap *et al*., 2016). It is clear from multiplex analysis detection that during the early stages of tumor development heterogeneity is pronounced (Bonavia *et al*., 2010). This shows that stem cells are causative agents for the initiation of breast cancer metastases. Paired-end sequencing is an exceptional technique that enables to study of the fusion of genres and it shows a unique expression of breast cancer stem cells. It is not possible with ordinary gene expression profiles which may provide information about tumor suppressor genes (Soon *et al*., 2013).

Variability was observed in patterns of gene expression when single cell RNA was used to profile five major glioblastomas of 430 cells. The variability was found primarily in various metabolic pathways and immune responses. Importantly, it was examined those glioblastomas contain a distinct population of cells that differs from stem cells slightly in their differentiation methods. The validity of this study can be checked by examining the gene expression profile (Patel *et al*., 2014).

***Breast cancer*.** SCS has been used to treat various types of tumors first report regarding the utilization of SCS in breast cancer was published in 2011. This report explained that when SCS of triple negative breast carcinomas was performed to check variation in copy number of flow sorted nuclei. It was found that one of the two tumors was made up of such cells which shows a single clonal expansion and is mono-genomic but on the other hand, the second carcinoma was genetically heterogeneous (Wang *et al*., 2014). It was reported that cells were taken from two breast cancer patients who were undergoing cell division to observe clonal diversity and mutations. It was found that the mutation rate is quite significant in triple negative breast tumor cells but when the genomic profiles of these cells were examined, they were not identical to any two cells (Gao *et al*., 2016).

It was assumed that point mutations gradually evolve over a longer period whereas alterations in copy number occur early in carcinogenesis. A follow up study was conducted in which 12 patients with triple negative breast cancer were considered as an experimental set. Single cell of 1000 cells was carried out and it was concluded that CNVs do not give rise to clonal populations and one out of every three sub-populations have the same evolutionary relationship (Zhang and Vijg, 2018). Similarly, it was reported that deletions and duplications occur too early in tumor development. In two patients who are (ER)-positive for breast cancer, single cell sequencing indicates the duplications of 1q and 8q and deletion of 11q regions. Using single cell sequencing it was suggested that early tumor development involves changes in copy number and point mutations and thus do not arise spontaneously rather they evolve gradually which generates an expansion of primary carcinoma (Baslan *et al*., 2015).

***Adenocarcinoma of the lung*.** The most common prevalent type of lung cancer is adenocarcinoma of the lung. The incidence rate of this subtype is greater than 40%. Various studies have been reported in which single cell RNA-seq has been performed on lung cancer patients. It was reported that 34 single cells were examined by using the PDX model. These cells were taken from lung adeno-carcinoma (Min *et al*., 2015). This results in the production of a set of 64 identified genes. The actual mechanism of the disease progression and diagnosis is not clear due to the affected cells are divided into two groups. In another study, mutation profiling was done and how these cells respond to anticancer treatments by RNA-sequence was evaluated (Kim *et al*., 2015). The combination of (KRAS) and G12D (35G>A) described that 69 genes are responsible

for this disease to develop and there is a mutation in all genes which contributes to lethality. It was also found that these cells can be divided into four major types each having its characteristic expression patterns. It is studied that a particular cell group showed inertness in its cell cycle. It is also found that transport genes are a major barrier in the treatment process as they are inert against chemotherapeutic agents. This study suggests while studying and analyzing large portions of primary carcinoma the actual causal agents for drug resistance may be masked but this limitation can be resolved using a single cell RNA sequence because it helps to detect potent drug-resistant clones. It is reported that seven patients affected by lung adeno-carcinoma were taken as samples and 336 cells were taken from the tumor sites to check how resistance to various drugs affected heterogeneity and up to which extent (Suzuki *et al*., 2015). It was found that two cell lines *i.e*. LC_2/ad cell line and (LC_2/ad-R) developed resistance against the drug vandetanib. When the reason for this resistance was investigated it was found that gene expression profiles showed profound changes in LC_2/ad-R cells than in LC_2/ad cells. As indicated in various studies great gene expression shows great diversification at the single cell level which is the reservoir for attaining drug resistance (Ichihara *et al*., 2009).

***Glioblastoma*.** Glioblastoma multiforme is the most common brain malignancy which is due to the formation of glial cells. These glial cells promote the growth of nerve cells and initial signs and symptoms are non-specific. Glioblastomas are biologically aggressive that are a major threat because they have an extremely fast proliferation rate throughout the brain and they are resistant to the conventional traditional as well as targeted therapies (Thakkar *et al*., 2014). EGFR is the major key player in the development of Glioblastoma. Extensive mutations take place in this gene and it results in the development of wild-type EGFR genes and other mutant types. Molecular heterogeneity also contributes to the development of mutant oncogenes. This in turn halts all over treatment procedures and corresponds to a lower survival rate. Tumor immunotherapy is a particular technique that is implied to various types of cancer and proven to be effective. To validate immunotherapy in the glioblastoma EGFR was amplified using WGS. It was found that copy number varies between single cells due to deletion, insertion, and mutations. So, it was concluded that this EGFR gene causes hindrance to drug response due to this particular variation (Francis *et al*., 2014). It is concluded that EGFR genes act differently in multiple cells and a combined therapy from multiple variants is needed to suppress the growth of tumors.

***Urinary system cancers*.** According to recent reports, bladder cancer is the most lethal form of cancer and it accounts for a 3% mortality rate worldwide and 5% in the United States (Saginala *et al*., 2020). Like GBM, heterogeneity is also observed in the single cells of bladder cancer patients as well as various infiltrating cells are also present (Lee *et al*., 2020). In a particular study, single-cell sequencing of 66 individual tumor cells was done and the data showed that they all have originated from a common ancestor but due to evolution they diverg 9e into various subtypes and perform function accordingly (Wang and Song, 2017). The authors hypothesized that during the developmental process, these cells have undergone selection pressure as well as different types of mutations. These mutations arise at a particular time during the development of cells and contribute to initiating carcinogenesis. This leads to resisting drug resistance (Lee *et al*., 2020; Wang and Song, 2017).

In another study, to investigate the level of heterogeneity 75 individual cells were subjected to RNA-sequencing in gene expression in multiple pathways, including MAPK, JAK-STAT and PI3K pathways (Zhao *et al*., 2021). These pathways proved to be very useful as they provide a deep insight into key targets for anticancer drugs. It is also clear that heterogeneity plays a key role in patient survival (Wu *et al*., 2014).

In another study, 20 single ccRCC cells were collected from a patient to observe intra-tumour heterogeneity. There is a rapid transition of normal cells to cancerous cells. There are very few mutations in cancer cells and these mutations were not identified previously using whole-tumor sequencing. It is also revealed from this study that renal carcinomas are genetically too complex as thought previously (Li *et al*., 2012).

During metastatic progression, various signaling pathways are activated which primarily affects the drug response. This patient did not show any response to conventional therapies. The results showed significant variability both in expression and pathway activation between primary carcinoma and metastasis. The variability was also observed among individual cancer cells in both tumors. It was mentioned previously that both EGFR and Src genes are heterogeneous and due

to this they are sensitive in their drug response. The molecular basis for treatment resistance was identified by transcription profiling and it was suggested that the combination of afatinib and asatinib would be fruitful (Zhang *et al*., 2016; Zhang *et al*., 2011).

Hematopoietic tumors. Hematopoietic and lymphoid tissue malignancies are too lethal and affect the blood, lymphatic system, and bone marrow. Hematopoietic cancers were initially studied on whole-genome sequencing of the tumor samples in bulk. In a research study by Hughes and colleagues, they sequenced a particular genotype of three patients and their genotype is composed of 1900 SNVs. These patients showed initial symptoms of myelodysplastic syndrome which developed into secondary acute myeloid leukemia (Adam *et al*., 2020). The single cell sequencing resolves the problem of clonal relationships as compared to the unfractionated tumor cells and also genomic complexity can be studied which was not clear with whole genome analysis. A study was carried out to observe the clonal structures and to understand the evolutionary history of acute lymphoblastic leukaemia (ALL). For this purpose, 1479 single cells were taken from six children suffering from pediatric ALL and targeted sequencing was performed to study deletions and structure of immunoglobulins (Hughes *et al*., 2014). It was found that like other carcinomas ALL carcinomas are composed of clonal cells which exhibit copy number variation followed by the development of SNVs. It was revealed that various clones expand to an abnormal degree and this expansion has a strong relationship with KRAS-associated driver mutations. Although due to expansion these clones achieve greater size as compared to other clones, they do not overshadow others as they continue to divide in the normal way. In a separate study single cell, WGS was performed in three children who have unstable B cell ALL to examine karyotype dynamics (Gawad *et al*., 2014).

At the time of diagnosis, traditional cytogenetics studies revealed that these carcinomas display different levels of aneuploidy. With traditional techniques, scientists are unable to perform comprehensive studies but with the help of SCS, such tumor populations which are not identified earlier and contain variations in their copy number were identified. It has been reported that ALL tumors show a different level of aneuploidy. To further validate this phenomenon a study was carried out within those tumor cells which exhibit intermediate aneuploidy. By inserting these cells in immunodeficient mice. It was observed that there is a difference in copy number which suggests that this heterogeneity may arise due to the response of cells to some external factors for example new environment (Bakker *et al*., 2016).

Essential thrombo-cythemia (ET) is a type of proliferative disease which occurs mostly in aged patients. In this condition, an abnormal number of platelets are produced in the body due to which various changes in body functions are observed. This disease arises due to mutation in various genes particularly (JAK2) gene thereby altering the normal JAK-STAT pathway. These mutations often lead to changes in the disease phenotype. To study the structure of clonal cells of diseased patients and the genes primarily involved, 58 single cells from a JAK2-negative ET patient were taken and WES was performed (Hou *et al*., 2012). Their results indicated the presence of 18 prominent genes which are playing a significant role in tumor development and they have a monoclonal origin. However, these conclusions do not correlate with phylogenetic analyses. Because according to phylogeny there is a large distance between cells. It is therefore unclear whether these differences arise due to real genomic diversity or they may arise due to technical defects (Macaulay and Voet, 2014).

It is not possible to study molecular heterogeneity in complex types of cancer. Also, this heterogeneous nature of cancerous cells cannot be studied with bulk tumor sequencing due to large data output. It is observed that SCS identifies most tumors that are polyclonal. Initially, in the development of such tumors changes in copy number were observed which changed to point mutations later. These point mutations are a major hurdle in the treatment process and affect the survival rate (Shepherd and Kent, 2019).

Brain tumor. It is generally a misconception that astrocytomas and oligo-dendrogliomas have a common origin (Ichihara *et al*., 2009). They both arise from glial cells in the brain and the major difference between them is difference in their genetics. There are also structural differences between them after the initiation of tumorigenesis. These both have similar tumor subtypes when studied through mass spectrometry. It also revealed that they arise from the same stem cells.

In human oligo-dendroglioma, single cell mapping has identified the same map for cancer stem cells and their progeny. The findings support the fact that stem cells are the chief source of mutations and the developmental

process. These studies suggest the presence of stem cells as new therapeutic targets. It has been found through research that immunotherapy is the main weapon to target specific cell types and therefore tumor growth can be suppressed. Hence, it proved to be highly significant for the treatment of such diseases (Perry, 2001).

***Head and neck cell carcinoma*.** Mostly the tumors which are found in the head and neck are squamous. These tumors develop in several places inside the head and neck and if they are diagnosed at early stages they are curable. Single cell sequencing has been a valuable source to study the micro-environment of tumors. This information is of utmost importance as it gives us an insight into the nature of the disease and how the disease progresses leading to a diseased state. It has also been observed that these carcinomas exhibit strong intra-tumor differences within a single population. Hence, SCS is of utmost importance as it provides detailed insight into the aberrations that are responsible for the heterogeneity in different cells. This leads to a combined effort to identify new biomarkers and the effects of targeted therapeutics in controlling the disease rate (Ang and Sturgis, 2012).

***Lung cancer and liver cancer*.** Lung cancer with liver metastasis refers to the type of cancer that affects the lungs initially and afterward crosses the blood barrier to reach the lymphatic system. SCS is quite effective in the treatment of this cancer. SCS provides insight into the liver micro-environment and the response of immune cells in response to metastases. In particular, comparative research by Chinese researchers, mapped the micro-environment of cancer affected liver and compared it with the T cell immune map of lung cancer (Ren *et al*., 2016). This mapping provides useful information about the heterogeneous nature of tumors and potent drug targets. It is therefore quite significant to find immuno-therapeutic agents in the liver and lungs micro-environment and in the discovery of novel biomarkers which are used to treat these malignancies (Wang *et al*., 2021).

***Single cell omics immunology*.** Immune response and immune functions are necessary for the body to resist invading external pathogens. There are a variety of immune cells each with distinguishing functions and they are hotspots of research.

***Application of natural killer (NK) cells, DC cells and lymphocytes*.** For detection to detect individual immune cells and their major group, single cell technologies are proved to be effective tools. These methods not only can detect individual cells but also distinguish different groups of these immune cells. Hence, they can provide information about the relationship among these immune cells. This is of primary importance as it helps to find new targets for the treatment of any disease by understanding the complex nature of immune cells (Guo *et al*., 2020). It is reported that natural killer cells are identified in various parts of the body. In a particular study, NK cells are identified in the spleen and blood of mice and humans. The results obtained provide a basis for the distinction between the spleen and blood NK cells.

The similarity between NK_1 and NK_2 cells in various organs is studied through the comparison of the transcriptome of both cells. This study provides a mechanism to cope with the various problem, particularly translational failure. This also provides a handful of information regarding the role and function of natural killer cells. In research studies, during the analysis of monocytes present in the human blood novel subtype of dendritic cells were also discovered. Later on, these dendritic cells were subjected to single cell RNA sequencing. It was also reported that these DC cells have properties similar to plasmacytoid. It was studied that DC cells activate T cells (Redmond *et al*., 2016). Many researchers proposed that to make the body's immune system strong, stimulation of these DC cells may be beneficial so that body can fight against invading pathogens. These cells are new agents to remove tumor cells because they have their immune system and chemotherapy may be prevented. The study also provides a comprehensive overview of the relationship among DC cells as well as the functions of the immune system in comparison to normal and diseased states and various other developmental processes. Single cell RNA sequencing is also used to study various cytokines and other immune cells which participate in the infection process. It was found various immune cells play a significant role in providing immunity against various infections, specifically IL-10 which expresses CD4 T cells (Guo *et al*., 2018).

***Causes of immune cell heterogeneity*.** Immune cells experience high heterogeneity due to invading pathogens. Single cell sequencing is used to study this heterogeneity and to study the genetic material of immune cells as well as the complex functions of immune cells in the body. Interestingly, it was also found that despite

pathogens ageing is also the main contributor to heterogeneity (Holzel *et al*., 2016). It is reported in a study that performing single-cell RNA sequencing transcription leads to elevated heterogeneity in gene expression among immune cells. It was also observed that immune cells are unable to perform their functions efficiently. This can cause the immune system to become weak with growing age. It is observed that ageing becomes more pronounced with the increase in intercellular transcriptional variation thereby this transcriptional variation provides a sound basis for discovering the mechanism of ageing.

It is also revealed from different studies that during autoimmune encephalomyelitis (EAE), antigens are mostly presented by dendritic cells and monocyte-derived cells. This study helped in mapping myeloid cell subpopulations and also revealed complex morphological changes in the central nervous system. This in turn a deep understanding of neuroinflammation and provides a sound basis for EAE treatment. It also states that the immune status of the body is badly affected by the heterogeneity of immune cells (Gerdes *et al*., 2014). The increase in the level of immune cell heterogeneity leads to decreased functionality of immune cells in fighting against invading pathogens which ultimately leads to a decline in overall immunity of the individual and the individual may become susceptible to various diseases. This is a sign of ageing. Hence the study of the heterogeneous nature of immune cells helps to understand the complex nature of immune cells but also provides a chance to adjust immune mechanisms to respond to various diseases (Trzupek *et al*., 2021).

Single cell transcriptome analysis reveals the discovery of new cell types in various systems of the body, especially the digestive system, for example, many intestinal cells are identified using this method. After the identification of these epithelial cells, mapping was done. The maps which are obtained help explain various functions of these epithelial cells, particularly in homeostasis and their response to pathogens (Levitin *et al*., 2018). It is reported that single cell transcriptome analysis also demonstrated the patterns of heterogeneity of precursor cells which form the initial nephron at an embryonic stage in humans (Lawson *et al*., 2018). Later, all events taking place during the development process may be analyzed using this SCS technique. It is of view that the differentiation process of precursor cells into tubular epithelial cells accompanies various changes in the signalling mechanisms and can be studied by using single cell transcriptome sequencing. This study also provides information about the authenticity of the candidate genes for congenital nephropathy and explains the mechanism of the treatment of this lethal disease (Tang *et al*., 2019).

Single cell omics in circulating tumor cells. Metastasis refers to the detachment of tumor cells from the primary tumor or metastatic sites and their entrance into the peripheral blood. These cells are called circulating tumor cells (CTCs). Likewise, primary tumors also show spatial and temporal heterogeneity. CTCs are tangled in the process of tumor progression so they help in studying the metastases process. The complex relationships that tumor cells have between them and also between the surrounding normal tissues can be studied using single cell omics analysis. However, despite being beneficial in providing information about tumor progression these CTCs also pose a serious threat to resisting therapy. This offers a negative impact on these CTCs and thus delimits their clinical practical efficacy (Lv *et al*., 2019).

As CTCs technologies are increasing each day and receiving immense success compared to omics methods but there is also a drawback that comparative analyses cannot be performed. The CTCs are present in rare numbers also there is no minimal criterion upon which we can refer to cells as CTCs. Also, comparative analysis cannot be performed using CTCs which represents a major challenge. Because of their movement in peripheral blood, they cannot be distinguished from normal blood cells. Hence, these CTCs are studied best when individual tumor profiles are provided (Jiang *et al*., 2015). They are also served as a source through which early detection of various kinds of tumors can be done. For interpreting data, the need of the hour is to create various computational tools. This can be done by the mutual efforts of biologists, bioengineers, and clinicians. These tools can be helpful to validate molecular data efficiently and management of precision medicine becomes easy for every single patient (Gkountela *et al*., 2019).

It has been studied that cancer proliferation depends upon the expansion of the clonal population which may lead to producing various molecular changes. This clonal expansion accelerates the process of metastasis and leads to therapeutic resistance. It is reported in numerous studies that mutational profiles are similar in primary carcinomas, metastases and CTCs when they

are identified by NGS. These profiles are for a variety of cancer types and in each profile, molecular heterogeneity is detected which shows that CTCs are valuable markers for cancer detection and patient care (Visal *et al*., 2022).

In a study, focusing on colorectal cancer specifically NGS analysis of 68 cancer genes is performed. It was concluded that mutations that are present in these genes are also found in CTCs. Hence, mutations are the chief source of the progression of metastasis in colorectal cancer. This information suggests that the CTCs are the chief source to determine the mutational spectrum of complex tumor genomes (Del Vecchio *et al*., 2017). Similarly, in another study, it has been observed that CTCs which are found in lung cancer patients are similar in the various metastatic sites within the same patient. Hence it is concluded that CTCs are mobile (Young *et al*., 2012).

Analysis of genomes of CTCs reveals the presence of mutational heterogeneity. It is also noted that further genomic changes promote metastasis in CTCs and DTCs. This information leads to the fact that mutational changes keep on going as the disease progress which results in a complex cancer genome. So, it is important to target these CTCs to control disease progression. Numerous genomic samples from a single cancer patient reveal the presence of CTCs and close examination of these CTCs and then designing a suitable therapeutic response is more effective as compared to traditional biopsies (Wiedswang *et al*., 2003).

In breast cancer patients, therapeutic strategies are highly dependent on the nature of CTCs and this nature can be modified by gene expression profiling. Despite genomic heterogeneity, it is noted that cell-to-cell variability is quite prominent between CTCs in the patterns of gene expression (Balic *et al*., 2006). It has also been reported that women suffering from negative breast cancer (-HER2) also develop positive type (+HER2). This may be illustrated by the fact that CTCs are mobile and RNA-of CTCs help provide comprehensive profiling. The discrete populations of HER2+ and HER2 – CTCs have persistent nature due to which they introvert quickly. This persistent nature is also a reason for acquiring drug resistance due to which disease progresses quickly. Similarly, heterogeneity is also present when single cell transcriptome analysis of CTCs was carried out which is the main driving source in the progression of metastasis and is eminent through changes in gene expression. It also leads to the induction of EMT, the process in which epithelial cells switch to a highly mesenchymal phenotype which leads to increased resistance against apoptosis (Fehm *et al*., 2010).

In men, prostate cancer is common lethality and it has also been studied through research that the androgen receptor plays a significant role in the development of this disease but in some cases, inertness to this receptor has been reported. This is because CTCs show heterogeneity in response to the expression of AR gene mutations when analyzed through single-cell RNA-seq of individual CTCs. This, in turn, may be proved critical to patient care as this can trigger the Wnt signaling pathway which accelerates the progress of the disease (Park *et al*., 2014). In pancreatic ductal adenocarcinoma, single intact cells from the primary carcinoma were compared with CTCs through expression profiles of RNA-sequin a mouse model of pancreatic cancer (Gall *et al*., 2019). According to this comparative study, certain specific cells are associated with stem cells and due to these genes expression of epithelial markers is masked. From mesenchymal transcripts, a high degree of heterogeneity was reported in CTCs. It has also been studied that this degree of heterogeneity is also seen in various blood markers and candidate genes (Keller and Pantel, 2019).

Disseminated tumor cells. It is evident that certain cells from CTCs disseminate from the primary carcinoma and result in the production of disseminated tumor cells DTCs. These DTCs contribute to speeding up the metastasis rate. DTCs are chiefly found in the bone marrow of cancer patients. In breast cancer patients it was found in a study that a single DTCs is present in the bone marrow and it is responsible for the survival of cancer cells and contributes to lethality (Carpenter *et al*., 2014). Patients which have non-metastatic breast cancer remain at greater risk because of DTCs which may contribute to the resurgence of cancer due to their persistent nature. This might happen also in the case of primary carcinoma has been removed (Bidard *et al*., 2008).

It is also reported that various DTCs present in bone marrow express proteins that are similar to cancer stem cells showing strong homology between them. This can be observed through the breast scanner (Slade and Coombes, 2007). Hence, it can also be predicted that DTCs originated from CTCs due to their strong similar

characteristics. They ascend from primary carcinoma and after dissemination, their physiology and molecular structure change (Niu *et al*., 2016). It is found that the genetic variation between CTCs and DTCs arises when the disease progresses. The cancer biomarkers between the two also differ accordingly. Significant molecular changes are noted also between the two which may arise due to the application of various therapeutic agents (Blackburn *et al*., 2015)

Because of the complicated surgical trials to collect DTCs practical utility of SCS is limited. It has been reported in a research study on a neuroblastoma patient that the same kind of mutation is present in different metastatic sites. The candidate gene anaplastic lymphoma kinase (ALK) gene was studied in primary tumor sites and DTCs in bone marrow. For this research 144 DTCs were also analyzed by single-cell WGA and it was concluded that mutations are the same in both sites (Carpenter *et al*., 2014). In another study, SCS was performed to trace the origin of 63 DTCs in breast cancer patients (Liu *et al*., 2017). Approximately about one-half of the DTCs have originated from the primary tumor site. Due to this reason, they have a strong morphological resemblance. However, other cells show variation in copy number profiles and some cells also have CNVs that are different from primary carcinoma genetically. If the evolutionary background of DTCs and the primary tumor is traced it is found that DTCs have arisen from a dominant clone in primary carcinoma and other forms of DTCs have originated from a primary tumor that is less prevalent. It is also reported that minor clones are present in lymph nodes and very few DTCs originate from it (Dasgupta *et al*., 2017).

Single cell omics in prenatal diagnosis. Single cell sequencing is recently used in the prenatal diagnosis of fatal diseases. SCS is widely used in pre-implantation genetic diagnosis (PGD) and non-invasive prenatal diagnosis (NIPD). The use of SCS in these diagnoses greatly increased the likelihood of healthy birth (Vora and Hui, 2018). Nowadays aneuploidy and SNPs are also detected using SCS. It is reported that the diagnosis of aneuploidy in embryo biopsy is easily done following WDA and it has maximum efficacy. In another study, aneuploidy was screened by using a single cell NGS-based technique in single blastomeres and it validates the previous study and shows high profile efficiency. In the subsequent study, the WDA protocol is compared with array comparative genomic hybridization (array-CGH) and it showed that visibility with the single cell NGS-based method is highly proficient. This proficiency can be categorized in terms of high throughput and reliability (Dondorp *et al*., 2015). Another study using single-cell stated that NGS is used to validate distributive patterns of segmental aneuploidies. This can be achieved by comparison of pure and mosaic segmental aneuploidies with that of CGH in trophectoderm biopsy (García-Pascual *et al*., 2020). In a particular study, 99 sperm sample was taken from an Asian male for the detection of SNPs and aneuploidy through MALBEC. This method was also followed in another study where oocytes were taken as a sample for the detection of SNPs and aneuploidy (Lu *et al*., 2012).

In addition to this, it is observed that the fetus may be affected by various means such as the placenta, environmental factors, etc. Affected fetuses can be detected using NIPD when used in combination with NGS technologies. It is now becoming a popular and safer tool to observe the affected fetuses as well as an interesting research topic (Hou *et al*., 2013). A subsequent study provides basic information regarding parallel sequencing for the detection of CNVs (Zhang *et al*., 2013). It is also reported that CNVs are 99.63% sensitive while aneuploidies are 97.71% specific. It is reported previously that fetal nucleated red blood cells were sequenced using WGA and Illumina MiSeq and out of 10 single-cells, aneuploidy was detected in 5 cases in placental villi (Hou *et al*., 2015).

Single cell omics in pulmonary diseases. Single cells have been used to study various pulmonary diseases such as asthma and Idiopathic pulmonary fibrosis (IPF) (Mazzei *et al*., 2019). The difference in the structure of DNA is a major risk factor in the treatment of rare and novel diseases. Because DNA is composed of non-cancerous somatic cells and the amount of DNA is nearly equal in all cells, a high quality sample of DNA can be obtained from peripheral blood at any stage of individual life and sequence variation can be measured across the genome (Kan *et al*., 2017). Asthma and COPD are studied by GWAS comparison of two cohorts, one cohort represents the European population and the other cohort represents the North American population. It is observed that an asthma signal is present at the 7q21 locus that includes the ORMDL3 and GSDMB genes and these genes promote the onset of asthma in adulthood. In another study, asthma associations are found in IL33, TSLP and IL1RL1 which supports the

fact that epithelial cytokines play a major role in the activation of T helper cells which promote asthma pathogenesis (Kim *et al*., 2013).

A cell or a tissue expresses various transcripts and their characterization depends upon many factors such as health status and developmental processes (Vaquerizas *et al*., 2009). The major goal of transcriptomics is to analyze specific cells or tissues in a controlled environment or diseased state to identify the major changes which lead to the development of specific biomarkers (Carraro and Stripp, 2022). Initially, transcriptomes were analyzed by microarray but after the discovery of NGS characterization was done with RNA-seqence. The site of extraction of a specific RNA sequence is critical. For lung diseases, RNA is extracted from either lung, bronchial epithelial tissues, nasal fluids or airway smooth muscle cells. Through transcriptome analysis, various virulent genes and pathways are identified which help to detect disease sub-phenotypes known as endotypes (Yates *et al*., 2014). In a particular study related to asthma, white blood cells were taken from 17 severe asthma patients, and their expression profiles were analyzed which indicate the presence of transduction receptors (TAS2Rs) which are expressed highly in severe asthma patients. This study proved transduction receptors (TAS2Rs) main causative agent of asthma and hence a potent drug target (Tsitsiou *et al*., 2012).

In another study, using RNA-sequencing transcriptome profiles of endobronchial biopsies of both asthma patients and control groups were analyzed and identified 46 expressed genes including SLC26A4, POSTN and BCL_2 but these results are not validated further (Yick *et al*., 2013). It is reported that transcriptome analysis of COPD patients shows a variation in gene expression. In particular, sputum was taken from 148 COPD patients and results indicate that extent of emphysema has increased with changes in gene expression and protein expression of airway macrophages also seems to be elevated (Singh *et al*., 2011). In another study, it was found that elevating respiratory stress leads to increased expression of neutrophil proteases (Almansa *et al*., 2012).

Various proteomics studies have been performed to identify biomarkers for ARDS but no significant results were found. In a recent metabolomics study regarding ARDS, undiluted pulmonary oedema was taken from ARDS patients which were compared to hydrostatic pulmonary oedema of control groups and results indicate the presence of an endotype with 235 metabolites which are major contributors to mortality rate (Rogers *et al*., 2017). Metabolomics studies were also performed for COPD to identify the primary markers which contribute to pathogenicity despite smoking status and identified chronic inflammation pathways (Diao *et al*., 2019).

Single cell omics in stem cell research. Stem cells can differentiate into specialized cells. Due to these characteristics, they have profound applications in developmental biology and regenerative medicine. It has been studied that embryonic and adult tissue stem cells are heterogeneous and are composed of multiple types and sub types. This heterogeneity can be masked by applying omics analysis and single cell approaches give a better insight into specific cell phenotypes and are also helpful in finding out variations between cells (Yan *et al*., 2013). It has been identified through single cell-RNA-seq that pluripotent genes show variation in human and mouse embryos. Single cell-RNA-seq also provides useful information about the tissue stem cells and the results showed that novel stem cell types are found by observing transcriptional profiles. It provides deep insight into various physiological processes which are taking place in normal and perturbed conditions (Deng *et al*., 2014).

Transcriptome profiles of mouse and human pre-implantation development have been conserved. It has been investigated that mostly all the cells of a single stage are the same except there are some inter-blastomere differences that take place at the fourth stage in the mouse. It has been confirmed that in the human zygote, the genome is activated in four-eight cell stages (Biase *et al*., 2014).

Embryonic stem cells. To study the self renewal nature and the developmental methods, human and mouse embryonic stem cells serve as excellent *in-vitro* models. By providing optimum conditions, it is possible to develop stem cells from blastocysts. It has been confirmed through these studies that the self-renewal process involves changes in the gene expression of transcriptional regulators that are associated with pluripotency (Blakeley *et al*., 2015). In another comparative study between human embryonic stem cells and EPI, it was investigated those various pathways are being involved and they are conserved for future studies. Human embryonic stem cells correspond to various signalling pathways including the WNT pathway,

MAPK pathway, and fibroblast growth factor pathway whereas, EPI corresponds to oxidative phosphorylation. It is clear from this study that human embryonic stem cells and EPI are different in many aspects and they need different mechanisms to maintain their pluripotent state (Dreesen and Brivanlou, 2007).

Primordial germ cells. The forerunners of germ cells *i.e*. sperms and oocytes are called primordial germ cells. It has been found that human PGCs show a balanced expression of pluripotent genes as well as germline genes when studied through single cell-RNA-seq. In the same embryo, PGCs are homogenous during mitosis whereas they show heterogeneous nature in early meiosis. A comparative study was also carried out between mice and humans to find out the differences between PGCs and it was found that early PGCs in humans express SOX15 and SOX17 whereas mice express Sox_2 (Petropoulos *et al*., 2016).

Tissue specific stem cells. In developing tissues stem cells are found and they are tissue specific. They are capable to differentiate into distinct structures. Recently single cell-RNA-seq methods are applied to tissue stem cells and identification of novel cell types and heterogeneity. To identify novel stem cell types a study on mouse lung epithelium was carried out using single cell-RNA-seq (Bryder *et al*., 2006). There are two types of epithelial cells in mouse lungs designated as type AT_1 cells and AT_2 cells which are involved in gaseous exchange. 80 individual epithelial cells from mouse lungs were taken and identification of five distinct cell populations was done. It is also studied that alveolar progenitors are bi-potential progenitors which give rise to AT_1 and AT_2 cells. RNA-sequencing data provide information about developmental intermediates and the complete mechanism of differentiation of bi-potential progenitors to alveolar cell types (Nabhan *et al*., 2018).

Single cell omics. *Role in the study of infectious diseases.* Infectious diseases are the chief source of mortality throughout the world. The incidence of infectious diseases is increasing day by day. To have deep insight into the mechanism of infection, it is significant to study host-pathogen interaction. This interaction is complex because immune cells vary from person to person and there is also variability in gene expression. The bulk analysis is not suited to study these interactions because they do not provide exact information regarding disease mechanisms. Single cell omics is widely used to study infectious diseases because analysis of variation among cells can be identified at the single cell level. Moreover, the single cell analysis provides insight into the host defence mechanism (Avraham *et al*., 2015).

Viruses cause several diseases in living species. Mostly, fluorescent techniques are used to study the disease mechanism in infected cells. The single cell analysis of various infected cells from viruses shows that there is a difference in the replication process. It is because different individuals consist of distinct cells and their response to pathogens is also different. In a particular Salmonella study in a mouse model, single cell RNA-seq was performed to evaluate the pathogenesis. RNA was labelled with particular fluorescent markers and these markers enable to identify of infected macrophages with dead bacteria and with living bacteria. Also, they provide information regarding uninfected macrophages. These results concluded that those macrophages which have dead bacteria harbour pro-inflammatory M_1 and those containing living bacteria differentiate into anti-inflammatory M_2 macrophages (Lin *et al*., 2020).

In infection models, single cell methods provide a detailed insight into the host-pathogen relationship and particular drug targets. Single cell-RNA-seq provides information regarding precise information about drug targets at an early stage of infection which leads to better treatment procedures and overall contributes to effective patient care (Luo *et al*., 2020). In addition to this, these methods also provide information about the relationship between immune cells and human microbiota which explains how cell functions are affected by human microbiota in the normal and pathological states (Cassotta *et al*., 2020). Due to these facts, single cell methods are effective tools for studying infectious diseases. However, there are certain limitations which include the small size of microorganisms which contributes to lower nucleic acid and protein content, and the high GC content of microbial genomes which hinder in sequencing process and effective single cell studies (Llorens-Rico *et al*., 2022).

Conclusion

Single cell omics have brought a revolution in cellular biology. Omics technology enabled different modalities of single cells such as transcriptomes, proteomes, and genomes. These brought high-resolution data about complex issues. Although these technologies have their

limitations, they provide a deep understanding of diseased tissues and phenotypes. This, in turn, proved to be an effective tool in the treatment of lethal diseases such as cancer.

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References

Adam, G., Rampášek, L., Safikhani, Z., Smirnov, P., Haibe-Kains, B., Goldenberg, A. 2020. Machine learning approaches to drug response prediction: challenges and recent progress. *NPJ Precision Oncology*, **4:** 1-10.

Akrap, N., Andersson, D., Bom, E., Gregersson, P., Ståhlberg, A., Landberg, G. 2016. Identification of distinct breast cancer stem cell populations based on single-cell analyses of functionally enriched stem and progenitor pools. *Stem Cell Reports*, **6:** 121-136.

Alix-Panabières, C., Pantel, K. 2013. Circulating tumor cells: liquid biopsy of cancer. *Clinical Chemistry*, **59:** 110-118.

Almansa, R., Socias, L., Sanchez-Garcia, M., Martín-Loeches, I., del Olmo, M., Andaluz-Ojeda, D., Bermejo-Martin, J.F. 2012. Critical COPD respiratory illness is linked to increased transcriptomic activity of neutrophil proteases genes. *BMC Research Notes*, **5:** 1-8.

Ang, K.K., Sturgis, E.M. 2012. Human papillomavirus as a marker of the natural history and response to therapy of head and neck squamous cell carcinoma. *Seminars in Radiation Oncology*, **22:** 128-142.

Avraham, R., Haseley, N., Brown, D., Penaranda, C., Jijon, H.B., Trombetta, J.J., Hung, D.T. 2015. Pathogen cell-to-cell variability drives heterogeneity in host immune responses. *Cell*, **162:** 1309-1321.

Bakker, B., Taudt, A., Belderbos, M. E., Porubsky, D., Spierings, D.C., de Jong, T.V., Foijer, F. 2016. Single-cell sequencing reveals karyotype heterogeneity in murine and human malignancie. *Genome Biology*, **17:** 1-15.

Balic, M., Lin, H., Young, L., Hawes, D., Giuliano, A., McNamara, G., Cote, R.J. 2006. Most early disseminated cancer cells detected in bone marrow of breast cancer patients have a putative breast cancer stem cell phenotype. *Clinical Cancer Research*, **12:** 5615-5621.

Baslan, T., Hicks, J. 2017. Unravelling biology and shifting paradigms in cancer with single cell sequencing. *Nature Reviews Cancer*, **17:** 557-569.

Baslan, T., Kendall, J., Ward, B., Cox, H., Leotta, A., Rodgers, L., Hicks, J. 2015. Optimizing sparse sequencing of single cells for highly multiplex copy number profiling. *Genome Research*, **25:** 714-724.

Biase, F., Cao, X., Zhong, S. 2014. Cell fate inclination within 2-cell and 4-cell mouse embryos revealed by single-cell RNA sequencing. *Genome Research*, **24:** 1787-1796.

Bidard, F.C., Vincent-Salomon, A., Gomme, S., Nos, C., de Rycke, Y., Thiery, J. P. 2008. Disseminated tumor cells of breast cancer patients: a strong prognostic factor for distant and local relapse. *Clinical Cancer Research*, **14:** 3306-3311.

Blakeley, P., Fogarty, N.M., Del Valle, I., Wamaitha, S.E., Hu, T.X., Elder, K., Niakan, K.K. 2015. Defining the three cell lineages of the human blastocyst by single cell RNA-seq. *Development*, **142:** 3151-3165.

Boman, B.M., Wicha, M.S. 2008. Cancer stem cells: a step toward the cure. *Journal Clinical Oncology*, **26:** 2795-2799.

Bonavia, R., Mukasa, A., Narita, Y., Sah, D.W., Vandenberg, S., Brennan, C., Furnari, F. 2010. Tumor heterogeneity is an active process maintained by a mutant EGFR-induced cytokine circuit in glioblastoma. *Genes and Development*, **24:** 1731-1745.

Bryder, D., Rossi, D.J., Weissman, I.L. 2006. Hematopoietic stem cells: the paradigmatic tissue-specific stem cell. *The American Journal of Pathology*, **169:** 338-346.

Blackburn, H., Schroeder, B., Turner, C.D., Shriver, C.L., Ellsworth, D., E Ellsworth, R. 2015. Management of incidental findings in the era of next-generation sequencing. *Current Genomics*, **16:** 159-174.

Carpenter, E.L., Rader, J., Ruden, J., Rappaport, E.F., Hunter, K.N., Hallberg, P.L., Mosse, Y.P. 2014. Dielectrophoretic capture and genetic analysis of single neuroblastoma tumor cells. *Frontiers in Oncology*, **4:** 201-214.

Carraro, G., Stripp, B.R. 2022. Insights gained in the

pathology of lung disease through single cell transcriptomics. *The Journal of Pathology*, **257:**494-500.

Cassotta, M., Forbes-Hernández, T.Y., Calderón Iglesias, R., Ruiz, R., Elexpuru Zabaleta, M., Giampieri, F., Battino, M. 2020. Links between nutrition, infectious diseases and microbiota: Emerging technologies and opportunities for human-focused research. *Nutrients*, **12:** 1827-1855.

Chen, W., Li, S., Kulkarni, A.S., Huang, L., Cao, J., Qian, K., Wan, J. 2020. Single cell omics: from assay design to biomedical application. *Biotechnology Journal*, **15:**1900262-1900272

Choi, J. R. 2020. Advances in single cell technologies in immunology. *Biotechniques*, **69:** 226-236.

Chung, W., Eum, H.H., Lee, H.O., Lee, K.M., Lee, H. B., Kim, K.T., Park, W.Y. 2017. Single cell RNA-seq enables comprehensive tumour and immune cell profiling in primary breast cancer. *Nature Communications*, **8:** 1-12.

Dasgupta, A., Lim, A.R., Ghajar, C.M. 2017. Circulating and disseminated tumor cells: harbingers or initiators of metastasis? *Molecular Oncology*, **11:** 40-61.

Del Vecchio, F., Mastroiaco, V., Di Marco, A., Compagnoni, C., Capece, D., Zazzeroni, F., Tessitore, A. 2017. Next-generation sequencing: recent applications to the analysis of colorectal cancer. *Journal of Translational Medicine*, **15:** 1-19.

Deng, Q., Ramskold, D., Reinius, B., Sandberg, R. 2014. Single-cell RNA-seq reveals dynamic, random monoallelic gene expression in mammalian cells. *Science*, **343:** 193-196.

Diao, W., Labaki, W.W., Han, M.K., Yeomans, L., Sun, Y., Smiley, Z., Stringer, K.A. 2019. Disruption of histidine and energy homeostasis in chronic obstructive pulmonary disease. *International Journal of Chronic Obstructive Pulmonary Disease*, **14:** 2015-2025.

Dondorp, W., De Wert, G., Bombard, Y., Bianchi, D. W., Bergmann, C., Borry, P., Cornel, M.C. 2015. Non-invasive prenatal testing for aneuploidy and beyond: challenges of responsible innovation in prenatal screening. *European Journal of Human Genetics*, **23:** 1438-1450.

Dontu, G., Al-Hajj, M., Abdallah, W.M., Clarke, M.F., Wicha, M.S. 2003. Stem cells in normal breast development and breast cancer. *Cell Proliferation*, **36:** 59-72.

Dreesen, O., Brivanlou, A. H. 2007. Signaling pathways in cancer and embryonic stem cells. *Stem Cell Reviews*, **3:** 7-17.

Dwivedi, S., Purohit, P., Misra, R., Lingeswaran, M., Vishnoi, J.R., Pareek, P., Misra, S. 2019. Application of single cell omics in breast cancer. *Indian Journal of Clinical Biochemistry*, **34:** 3-180.

Fehm, T., Müller, V., Aktas, B., Janni, W., Schneeweiss, A., Stickeler, E., Pantel, K. 2010. HER2 status of circulating tumor cells in patients with metastatic breast cancer: a prospective, multicenter trial. *Breast Cancer Research and Treatment*, **124:** 403-412.

Francis, J.M., Zhang, C.Z., Maire, C.L., Jung, J., Manzo, V.E., Adalsteinsson, V.A., Ligon, K.L. 2014. EGFR variant heterogeneity in glioblastoma resolved through single-nucleus sequencing single-nucleus sequencing of GBM mutational heterogeneity. *Cancer Discovery*, **4:** 956-971.

Gajecka, M. 2016. Unrevealed mosaicism in the next-generation sequencing era. *Molecular Genetics and Genomics*, **291:** 513-530.

Gall, T.M., Belete, S., Khanderia, E., Frampton, A.E., Jiao, L.R. 2019. Circulating tumor cells and cell-free DNA in pancreatic ductal adenocarcinoma. *The American Journal of Pathology*, **189:** 71-81.

Gao, R., Davis, A., McDonald, T.O., Sei, E., Shi, X., Wang, Y., Navin, N.E. 2016. Punctuated copy number evolution and clonal stasis in triple-negative breast cancer. *Nature Genetics*, **48:** 1119-1130.

García-Pascual, C.M., Navarro-Sánchez, L., Navarro, R., Martínez, L., Jiménez, J., Rodrigo, L., Rubio, C. 2020. Optimized NGS approach for detection of aneuploidies and mosaicism in PGT-A and Imbalances in PGT-SR. *Genes*, **11:** 724-735.

Gawad, C., Koh, W., Quake, S.R. 2014. Dissecting the clonal origins of childhood acute lymphoblastic leukemia by single-cell genomics. *Proceedings of the National Academy of Sciences*, **111:** 17947-17952.

Gerdes, M.J., Sood, A., Sevinsky, C., Pris, A.D., Zavodszky, M.I., Ginty, F. 2014. Emerging understanding of multiscale tumor heterogeneity. *Frontiers in Oncology*, **4:** 366-378.

Gkountela, S., Castro-Giner, F., Szczerba, B.M., Vetter, M., Landin, J., Scherrer, R., Aceto, N. 2019. Circulating tumor cell clustering shapes DNA methylation to enable metastasis seeding. *Cell*, **176:** 98-112.

Guo, T., Li, W., Cai, X. 2020. Applications of single cell omics to dissect tumor microenvironment. *Frontiers in Genetics*, **11:** 548719-548739.

Guo, X., Zhang, Y., Zheng, L., Zheng, C., Song, J.,

Zhang, Q., Zhang, Z. 2018. Global characterization of T cells in non-small-cell lung cancer by single cell sequencing. *Nature Medicine*, **24:** 978-985.

Hardin, M., Silverman, E.K. 2014. Chronic obstructive pulmonary disease genetics: a review of the past and a look into the future. *Chronic Obstructive Pulmonary Diseases: Journal of the COPD Foundation*, **6:** 33-46.

Helleday, T., Eshtad, S., Nik-Zainal, S. 2014. Mechanisms underlying mutational signatures in human cancers. *Nature Reviews Genetics*, **15:** 585-598.

Hölzel, M., Bovier, A., Tüting, T. 2016. Plasticity of tumour and immune cells: a source of heterogeneity and a cause for therapy resistance. *Nature Reviews Cancer*, **13:** 365-376.

Hou, Y., Fan, W., Yan, L., Li, R., Lian, Y., Huang, J., Qiao, J. 2013. Genome analyses of single human oocytes. *Cell*, **155:** 1492-1506.

Hou, Y., Song, L., Zhu, P., Zhang, B., Tao, Y., Xu, X., Wang, J. 2012. Single cell exome sequencing and monoclonal evolution of a JAK2-negative myeloproliferative neoplasm. *Cell*, **148:** 873-885.

Hou, Y., Wu, K., Shi, X., Li, F., Song, L., Wu, H., Wang, J. 2015. Comparison of variations detection between whole-genome amplification methods used in single cell resequencing. *Gigascience*, **4:** 13742-015.

Hu, Y., An, Q., Sheu, K., Trejo, B., Fan, S., Guo, Y. 2018. Single cell multi-omics technology: methodology and application. *Frontiers in Cell and Developmental Biology*, **6:** 28-41.

Hughes, A.E., Magrini, V., Demeter, R., Miller, C.A., Fulton, R., Fulton, L.L., Graubert, T.A. 2014. Clonal architecture of secondary acute myeloid leukemia defined by single cell sequencing. *PLoS Genet*, **10:** 1004462-10044474.

Ichihara, E., Ohashi, K., Takigawa, N., Osawa, M., Ogino, A., Tanimoto, M., Kiura, K. 2009. Effects of vandetanib on lung adenocarcinoma cells harboring epidermal growth factor receptor T790M mutation *in vivo*. *Cancer Research*, **69:** 5091-5098.

Jiang, R., Lu, Y.T., Ho, H., Li, B., Chen, J.F., Lin, M., Posadas, E.M. 2015. A comparison of isolated circulating tumor cells and tissue biopsies using whole-genome sequencing in prostate cancer. *Oncotarget*, **6:** 44781-44793.

Kan, M., Shumyatcher, M., Himes, B.E. 2017. Using omics approaches to understand pulmonary diseases. *Respiratory Research*, **18:** 1-20.

Keller, L., Pantel, K. 2019. Unravelling tumour heterogeneity by single-cell profiling of circulating tumour cells. *Nature Reviews Cancer*, **19:** 553-567.

Kim, K.T., Lee, H. W., Lee, H.O., Kim, S.C., Seo, Y.J., Chung, W., Park, W.Y. 2015. Single cell mRNA sequencing identifies subclonal heterogeneity in anti-cancer drug responses of lung adenocarcinoma cells. *Genome Biology*, **16:**1-15.

Kim, T.B., Jang, A.S., Kwon, H.S., Park, J.S., Chang, Y.S., Cho, S.H., Park, C.S. 2013. Identification of asthma clusters in two independent Korean adult asthma cohorts. *European Respiratory Journal*, **41:** 1308-1314.

Lawson, D.A., Bhakta, N.R., Kessenbrock, K., Prummel, K.D., Yu, Y., Takai, K., Werb, Z. 2015. Single-cell analysis reveals a stem-cell program in human metastatic breast cancer cells. *Nature*, **526:** 131-135.

Lawson, D.A., Kessenbrock, K., Davis, R.T., Pervolarakis, N., Werb, Z. 2018. Tumour heterogeneity and metastasis at single-cell resolution. *Nature cell biology*, **20:** 1349-1360.

Lee, H.W., Chung, W., Lee, H.O., Jeong, D.E., Jo, A., Lim, J.E., Park, W.Y. 2020. Single cell RNA sequencing reveals the tumor micro-environment and facilitates strategic choices to circumvent treatment failure in a chemorefractory bladder cancer patient. *Genome Medicine*, **12:**1-21.

Levitin, H.M., Yuan, J., Sims, P.A. 2018. Single cell transcriptomic analysis of tumor heterogeneity. *Trends in Cancer*, **4:** 264-268.

Li, H., Horns, F., Wu, B., Xie, Q., Li, J., Li, T., Luo, L. 2017. Classifying drosophila olfactory projection neuron subtypes by single cell RNA sequencing. *Cell*, **171:** 1206-1220.

Li, Y., Xu, X., Song, L., Hou, Y., Li, Z., Tsang, S., Wang, J. 2012. Single cell sequencing analysis characterizes common and cell-lineage-specific mutations in a muscle-invasive bladder cancer. *Giga Science*, **1:** 12-26.

Lin, W.N., Tay, M.Z., Lu, R., Liu, Y., Chen, C.H., Cheow, L.F. 2020. The role of single cell technology in the study and control of infectious diseases. *Cells*, **9:** 1440-1468.

Liu, J., Adhav, R., Xu, X. 2017. Current progresses of single cell DNA sequencing in breast cancer research. *International Journal of Biological Sciences*, **13:** 949-960.

Liu, J., Qu, S., Zhang, T., Gao, Y., Shi, H., Song, K., Yin, W. 2021. Applications of single cell omics in tumor immunology. *Frontiers in Immunology*, **12:** 697412-697424.

Lloréns-Rico, V., Simcock, J.A., Huys, G.R., Raes, J. 2022. Single cell approaches in human microbiome research. *Cell*, **185:** 2725-2738.

Lu, S., Zong, C., Fan, W., Yang, M., Li, J., Chapman, A.R., Xie, X. S. 2012. Probing meiotic recombination and aneuploidy of single sperm cells by whole-genome sequencing. *Science*, **338:** 1627-1630.

Luo, G., Gao, Q., Zhang, S., Yan, B. 2020. Probing infectious disease by single cell RNA sequencing: Progresses and perspectives. *Computational and Structural Biotechnology Journal*, **18:** 2962-2971.

Lv, Q., Gong, L., Zhang, T., Ye, J., Chai, L., Ni, C., Mao, Y. 2019. Prognostic value of circulating tumor cells in metastatic breast cancer: a systemic review and meta-analysis. *Clinical and Translational Oncology*, **18:** 322-330.

Macaulay, I.C., Voet, T. 2014. Single cell genomics: advances and future perspectives. *PLoS Genetics*. **10:** e1004126-e1004135.

Mazzei, M.A., Sartorelli, P., Bagnacci, G., Gentili, F., Sisinni, A.G., Fausto, A., Volterrani, L. 2019. Occupational lung diseases: underreported diagnosis in radiological practice. *Semi in Ultrasound, CT and MRI*, **40:** 36-50.

Min, J.W., Kim, W.J., Han, J.A., Jung, Y. J., Kim, K. T., Park, W.Y., Choi, S.S. 2015. Identification of distinct tumor subpopulations in lung adenocarcinoma *via* single-cell RNA-seq. *PloS One*, **10:** 013587-0135104.

Mincarelli, L., Mincarelli, L., Lister, A., Lipscombe, J., Macaulay, I.C. 2018. Defining cell identity with single cell omics. *Proteomics*, **18:** 1700312-1700329.

Nabhan, A.N., Brownfield, D.G., Harbury, P.B., Krasnow, M.A., Desai, T.J. 2018. Single cell Wnt signaling niches maintain stemness of alveolar type 2 cells. *Science*, **359:** 1118-1123.

Navin, N., Kendall, J., Troge, J., Andrews, P., Rodgers, L., McIndoo, J., Wigler, M. 2011. Tumour evolution inferred by single cell sequencing. *Nature*, **472:** 90-94.

Niu, F., Wang, D.C., Lu, J., Wu, W., Wang, X. 2016. Potentials of single cell biology in identification and validation of disease biomarkers. *Journal of Cellular and Molecular Medicine*, **20:** 1789-1795.

Pan, X. 2014. Single cell analysis: from technology to biology and medicine. *Single Cell Biology*, **3:** 106-116.

Park, S., Ang, R.R., Duffy, S.P., Bazov, J., Chi, K.N., Black, P.C., Ma, H. 2014. Morphological differences between circulating tumor cells from prostate cancer patients and cultured prostate cancer cells. *PloS One*, **9:** e85264-e852671.

Patel, A.P., Tirosh, I., Trombetta, J.J., Shalek, A.K., Gillespie, S.M., Wakimoto, H., Bernstein, B.E. 2014. Single cell RNA-seq highlights intratumoral heterogeneity in primary glioblastoma. *Science*, **344:** 1396-1401.

Paulsen, R.D., Soni, D.V., Wollman, R., Hahn, A. T., Yee, M.C., Guan, A., Cimprich, K.A. 2009. A genome-wide siRNA screen reveals diverse cellular processes and pathways that mediate genome stability. *Molecular Cell*, **35:** 228-239.

Peng, A., Mao, X., Zhong, J., Fan, S., Hu, Y. 2020. Single cell multi-omics and its prospective application in cancer biology. *Proteomics*, **20:** 1900271-1900280.

Perry, A. 2001. Oligodendroglial neoplasms: current concepts, misconceptions, and folklore. *Advances in Anatomic Pathology*, **28:** 183-199.

Petropoulos, S., Panula, S.P., Schell, J.P., Lanner, F. 2016. Single cell RNA sequencing: revealing human pre-implantation development, pluripotency and germline development. *Journal of Internal Medicine*, **280:** 252-264.

Redmond, D., Poran, A., Elemento, O. 2016. Single cell TCRseq: paired recovery of entire T-cell alpha and beta chain transcripts in T-cell receptors from single cell RNAseq. *Genome Medicine*, **8:** 1-12.

Ren, Y., Dai, C., Zheng, H., Zhou, F., She, Y., Jiang, G., Chen, C. 2016. Prognostic effect of liver metastasis in lung cancer patients with distant metastasis. *Oncotarget*, **7:** 53245-53253.

Riethdorf, S., Pantel, K. 2008. Disseminated tumor cells in bone marrow and circulating tumor cells in blood of breast cancer patients: current state of detection and characterization. *Pathobiology*, **75:** 140-148.

Rogers, A.J., Contrepois, K., Wu, M., Zheng, M., Peltz, G., Ware, L.B., Matthay, M.A. 2017. Profiling of ARDS pulmonary esdema fluid identifies a metabolically distinct subset. *American Journal of Physiology-Lung Cellular and Molecular Physiology*, **312:** 703-709.

Saginala, K., Barsouk, A., Aluru, J.S., Rawla, P., Padala, S.A., Barsouk, A. 2020. Epidemiology of bladder cancer. *Medical Sciences*, **8:** 15-27.

Shepherd, M.S., Kent, D.G. 2019. Emerging single cell tools are primed to reveal functional and molecular heterogeneity in malignant hematopoietic stem cells. *Current Opinion in Hematology*, **26:** 214-221.

Singh, D., Fox, S.M., Tal-Singer, R., Plumb, J., Bates,

S., Broad, P., Riley, J.H., Celli, B., Investigators, E. 2011. Induced sputum genes associated with spirometric and radiological disease severity in COPD ex-smokers. *Thorax*, **66:** 489-95.

Slade, M.J., Coombes, R.C. 2007. The clinical significance of disseminated tumor cells in breast cancer. *Nature Clinical Practice Oncology*, **4:** 30-41.

Soon, W.W., Hariharan, M., Snyder, M.P. 2013. High-throughput sequencing for biology and medicine. *Molecular Systems Biology*, **9:** 640-654.

Suzuki, A., Matsushima, K., Makinoshima, H., Sugano, S., Kohno, T., Tsuchihara, K., Suzuki, Y. 2015. Single cell analysis of lung adenocarcinoma cell lines reveals diverse expression patterns of individual cells invoked by a molecular target drug treatment. *Genome Biology*, **16:** 1-17.

Tang, X., Huang, Y., Lei, J., Luo, H., Zhu, X. 2019. The single-cell sequencing: new developments and medical applications. *Cell and Bioscience*, **9:** 1-9.

Thakkar, J.P., Dolecek, T.A., Horbinski, C., Ostrom, Q.T., Lightner, D.D., Barnholtz-Sloan, J.S., Villano, J.L. 2014. Epidemiologic and molecular prognostic review of glioblastoma GBM epidemiology and biomarkers. *Cancer Epidemiology*, *Biomarkers and Prevention*, **23:** 1985-1996.

Tian, Y., Carpp, L.N., Miller, H.E., Zager, M., Newell, E.W., Gottardo, R. 2022. Single cell immunology of SARS-CoV-2 infection. *Nature Biotechnology*, **40:** 30-41.

Trzupek, D., Lee, M., Hamey, F., Wicker, L.S., Todd, J.A., Ferreira, R.C. 2021. Single-cell multi-omics analysis reveals IFN-driven alterations in T lymphocytes and natural killer cells in systemic lupus erythematosus. *Wellcome Open Research*, **6:** 149-176.

Tsitsiou, E., Williams, A.E., Moschos, S.A., Patel, K., Rossios, C., Jiang, X., Lindsay, M.A. 2012. Transcriptome analysis shows activation of circulating CD8+ T cells in patients with severe asthma. *Journal of Allergy and Clinical Immunology*, **129:** 95-103.

Vaquerizas, J.M., Kummerfeld, S.K., Teichmann, S.A., Luscombe, N.M. 2009. A census of human transcription factors: function, expression and evolution. *Nature Reviews Genetics*, **10:** 252-263.

Visal, T.H., den Hollander, P., Cristofanilli, M., Mani, S.A. 2022. Circulating tumour cells in the-omics era: how far are we from achieving the 'singularity'? *British Journal of Cancer*, **127:** 173-184.

Vora, N.L., Hui, L. 2018. Next-generation sequencing and prenatal'omics: advanced diagnostics and new insights into human development. *Genetics in Medicine*, **20:** 791-799.

Wang, H., Meng, D., Guo, H., Sun, C., Chen, P., Jiang, M., He, Y. 2021. Single Cell Sequencing, an Advanced Technology in Lung Cancer Research. *OncoTargets and Therapy*, **14:** 1895-1909.

Wang, P., Chen, Y., Yong, J., Cui, Y., Wang, R., Wen, L., Tang, F. 2018. Dissecting the global dynamic molecular profiles of human fetal kidney development by single cell RNA sequencing. *Cell Reports*, **24:** 3554-3567.

Wang, J., Song, Y. 2017. Single cell sequencing: a distinct new field. *Clinical and Translational Medicine*, **6:** 1-11.

Wang, Y., Navin, N.E. 2015. Advances and applications of single cell sequencing technologies. *Molecular Cell*, **58:** 598-609.

Wang, Y., Waters, J., Leung, M.L., Unruh, A., Roh, W., Shi, X., Navin, N.E. 2014. Clonal evolution in breast cancer revealed by single nucleus genome sequencing. *Nature*, **512:** 155-160.

Ward-Hartstonge, K.A., Kemp, R.A. 2017. Regulatory T-cell heterogeneity and the cancer immune response. *Clinical and Translational Immunology*, **6:** 154-159.

Wicha, M.S., Liu, S., Dontu, G. 2006. Cancer stem cells: an old idea—a paradigm shift. *Cancer Research*, **66:** 1883-1890.

Wiedswang, G., Borgen, E., Karesen, R., Kvalheim, G., Nesland, J.M., Qvist, H., Naume, B. 2003. Detection of isolated tumor cells in bone marrow is an independent prognostic factor in breast cancer. *Journal of Clinical Oncology*, **21:** 3469-347.

Wu, A.R., Neff, N.F., Kalisky, T., Dalerba, P., Treutlein, B., Rothenberg, M.E., Quake, S.R. 2014. Quantitative assessment of single cell RNA-sequencing methods. *Nature Methods*, **11:** 41-46.

Yan, L., Yang, M., Guo, H., Yang, L., Wu, J., Li, R., Tang, F. 2013. Single cell RNA-seq profiling of human preimplantation embryos and embryonic stem cells. *Nature Structural and Molecular Biology*, **20:** 1131-1139.

Yates, S.A., Swain, M.T., Hegarty, M.J., Chernukin, I., Lowe, M., Allison, G.G., Skøt, L. 2014. De novo assembly of red clover transcriptome based on RNA-seq data provides insight into drought response, gene discovery and marker identification. *BMC Genomics*, **15:** 1-15.

Yick, C.Y., Zwinderman, A.H., Kunst, P.W., Grünberg, K., Mauad, T., Dijkhuis, A., Sterk, P.J. 2013. Transcriptome sequencing (RNA-Seq) of human endobronchial biopsies: asthma versus controls. *European Respiratory Journal*, **42:** 662-670.

Young, R., Pailler, E., Billiot, F., Drusch, F., Barthelemy, A., Oulhen, M., Vielh, P. 2012. Circulating tumor cells in lung cancer. *Acta cytologica*, **56:** 655-660.

Zhang, C., Zhang, C., Chen, S., Yin, X., Pan, X., Lin, G., Wang, W. 2013. A single cell level-based method for copy number variation analysis by low coverage massively parallel sequencing. *PloS One*, **8:** 54236-54245.

Zhang, L., Vijg, J. 2018. Somatic mutagenesis in mammals and its implications for human disease and aging. *Annual review of genetics*, **23:** 397-419.

Zhang, X., Marjani, S.L., Hu, Z., Weissman, S.M., Pan, X., Wu, S. 2016. Single cell sequencing for precise cancer research: progress and prospects single cell sequencing of cancer. *Cancer Research*, **76:** 1305-1312.

Zhang, X., Zhang, M., Hou, Y., Xu, L., Li, W., Zou, Z., Wu, S. 2016. Single-cell analyses of transcriptional heterogeneity in squamous cell carcinoma of urinary bladder. *Oncotarget*, **7:** 66069-66076.

Zhang, S., Huang, W.C., Li, P., Guo, H., Poh, S.B., Brady, S.W., Yu, D. 2011. Combating trastuzumab resistance by targeting SRC, a common node downstream of multiple resistance pathways. *Nature Medicine*, **17:** 461-469.

Zhao, Y., Li, Z. X., Zhu, Y.J., Fu, J., Zhao, X.F., Zhang, Y.N., Chen, L. 2021. Single cell transcriptome analysis uncovers intratumoral heterogeneity and underlying mechanisms for drug resistance in hepatobiliary tumor organoids. *Advanced Science*, **28:** 2003897-20038110.