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Artículo de revisión

Acute myelogenous leukemia: A perspective

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RESUMEN

La leucemia mieloblástica aguda se caracteriza por la extrema proliferación clonal de precursores hematopoyéticos indiferenciados o con diferenciación anormal. La quimioterapia está dirigida a la reducción y erradicación de células leucémicas. Sin embargo, generalmente se asume que la recaída ocurre gracias a la coexistencia de células residuales, que son resistentes o eluden la terapia. Durante los últimos años, la hipótesis de la célula madre cancerosa ha ganado considerable importancia y podría interpretar este comportamiento. Esta teoría persuasiva establece que dentro de un tumor, las células están organizadas en una jerarquía similar a la encontrada en los tejidos normales y son mantenidas por un pequeño conjunto de células responsables de la latencia del tumor. Estas células, definidas como "células iniciadoras de tumor" poseen varias propiedades de células madre de tejidos normales. Hace poco se demostró que las "células iniciadoras de tumor" asociadas con la leucemia mieloblástica aguda forman una clase distinta en una jerarquía similar a la observada en las células madre hematopoyéticas. Ahora sabemos que en la leucemia mieloblástica aguda el crecimiento y supervivencia de los blastos se lleva acabo por los mismos factores de crecimiento que estimulan a las células normales. Más aun, se ha puesto en evidencia el papel que juega el factor de crecimiento de la célula madre de tipo membranal y su receptor c-Kit en las interacciones célula-célula y en la supervivencia de dichos blastos, definiendo la importancia de la estimulación yuxtacrina. La inhibición de la transducción de señales de c-Kit induce combinaciones de muerte celular: autofagia (mecanismo compensatorio hacia la supervivencia) y apoptosis. Los inhibidores de c-Kit no sólo reducen la proliferación de células cancerosas sino que también pueden, usados inapropiadamente, deteriorar tejidos normales. El propósito de este artículo es revisar algunas de las características importantes de los blastos leucémicos en vista de la investigación realizada. En lugar de presentar detalles de varios estudios se intentan indicar áreas generales de trabajo realizado o en evolución. Se espera que este ensayo muestre otras oportunidades de investigación en la leucemia mieloblástica aguda.
Palabras clave: leucemia mieloblástica aguda, células madre cancerosas, autofagia, apoptosis.

ABSTRACT

Acute myeloblastic leukaemia is characterized by the extreme clonal proliferation of haematopoietic precursor cells with abnormal or arrested differentiation. Chemotherapy of acute leukaemia is channelled towards the reduction and eradication of leukaemic cells. However, relapse is generally assumed to occur in residual host cells, which are refractory to or elude therapy. The cancer stem cell hypothesis has gained considerable importance in recent years and could interpret this behaviour. This persuasive theory states that cells within a tumour are organized in a hierarchy similar to that of normal tissues, and are maintained by a small subset of cells responsible for tumour dormancy. These cells, defined as «tumour initiating cells» (TICs), possess several properties of normal tissue stem cells. Recently, the TICs associated with AML have been shown to comprise distinct, hierarchically arranged classes similar to those observed for haematopoietic stem cells. We know now that the growth and survival of blasts in AML are driven by the same growth factors that stimulate normal cells. Furthermore, direct evidence of the role of membrane steel factor and its receptor c-Kit in cell-cell interactions and cell survival in primary AML blasts has been provided, defining the importance of juxtacrine stimulation. Inhibition of c-Kit signaling induces combinations of cell death: autophagy (compensatory mechanism toward survival) and apoptosis. While recent work confirmed that c-Kit inhibitors reduce cancer cell proliferation, it also demonstrated that future inappropriate prescriptions could cause normal tissue deterioration. The purpose of this paper was to review some of the salient features of leukaemic blasts in support of the proposal that research into neoplasia be increased. Rather than presenting the details of various studies, I have attempted to indicate general areas in which work has been done or is in progress. It is hoped that this survey of the subject will demonstrate a variety of opportunities for additional research in human neoplasia.
Key words: AML, cancer stem cells, autophagy, apoptosis

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Acute myeloid leukaemia (AML) is a heterogeneous disorder

Acute myeloid leukaemia (AML) results in a lethal overgrowth of myeloid progeny in bone marrow. Nevertheless, the origin of cancer is veiled in obscurity. It would appear that, for a certain period, the constitution is able to withstand the development of cancer, until something causes the vital energy to be lowered, whereupon the slightest external injury is sufficient to accelerate the nascent germ and rouse it into activity. When these abnormal, or leukaemic, cells build up in the bone marrow and blood, there is less room for healthy white blood cells, red blood cells and platelets, which may lead to infection, anaemia, or easy bleeding. The leukaemia cells can spread outside the blood to other parts of the body, including the brain, skin and gums. Acute myeloid leukaemia (AML) remains one of the most difficult haematologic malignancies to treat. Improvements in therapy will come from the identification of new molecularly targeted agents. Clinically, AML has been recognized as a heterogeneous disorder,[1] although it results from two profound disturbances in haematopoiesis: a gain-of-function in proliferation and a loss-of-function in differentiation.[2] Typically, AML cells replace most of the normal haematopoietic lineages and lead to bone marrow failure and death from infection and/or haemorrhage.[3] Other features about AML include:[4,5]

Acute myeloid leukaemia comprises about 40% of leukaemias in the Western world. Approximately 6500 cases are diagnosed in adults in the US annually. The estimated incidence of AML in males and females in Mexico is approximately half of that from Surveillance, Epidemiology and End Results (SEER) data in the United States (6,7).

- Acute myeloid leukaemia is the most common type of blood cancer in adults, with a prevalence of 3-8 cases per 100,000, which rises to 17-19 per 100,000 in those aged 65 and older. If untreated, this form of leukaemia usually progresses quickly.
- An increased incidence of Acute myeloid leukaemia is associated with certain congenital chromosomal abnormalities (Down's syndrome, Bloom's syndrome, Fanconi's anaemia).
- Patients with acquired diseases such as myelodysplastic syndromes, myeloproliferative disorders and other pre-leukaemic states have an increased incidence of AML.
- A variety of environmental factors, both work and treatment related, are known to cause AML. These include:
  - Exposure to ionizing radiation;
  - Chemical exposure to benzene, and possibly hydrocarbons and solvents treatment with alkylating agents (e.g., melphalan, methyl CCNU(1-(2-Chloroethyl)-3-(4-methylcyclohexyl)-1-nitrosourea), nitrogen mustard) or procarbazine;
  - Treatment with other drugs (e.g., chloramphenicol, phenylbutozone).
- Another theory regarding the cause of AML is that of a viral cause.
- Acute myeloid leukaemia blast cells are often characterized by several adverse variables that predict poor treatment outcome, including high CD34 expression, minimally or undifferentiated features, high P-glycoprotein expression, a high bcl-2/bax ratio, an unfavourable karyotype and more frequent internal tandem duplications (ITDs) and mutations of class III receptor-type tyrosine kinase for key haematopoietic cytokines: Flt-3 (Flt-ligand receptor), c-Kit (steel factor receptor) and fms (M-CSF receptor) [8]. Although we now know a great deal about the adverse variables in AML, there is still much more to be learned to improve this knowledge.

The heterogeneity of Acute myeloid leukaemia cannot always be recognized by morphology, but it can be reflected by the underlying genetic aberrations. Since 2001, the World Health Organization (WHO) classification of myeloid neoplasms has been oriented towards categorizing disease entities according to their underlying genetic alterations. The new classification of acute myeloid leukaemia and precursor-related neoplasms (2008) is shown in Table 1.[9,10]

Growth and survival of blasts in AML are driven by the same growth factors that stimulate normal cells

It can be conservatively said that there are some AML-CFUs that possesses self-renewal capacity, a proliferation capacity with colony formation *in vitro* that can either differentiate spontaneously in suspension culture or when induced by appropriate inducing agents; all of these properties fulfil the characteristics of a stem cell. Nevertheless, only a small proportion of AML cells are clonogenic

Table 1. Acute myeloid leukaemia and related precursor neoplasia

Acute myeloid leukaemia with recurrent genetic abnormalities
Acute myeloid leukaemia with t(8;21)(q22;q22); RUNX1-RUNX1T1
Acute myeloid leukaemia with inv(16)(p13.1q22) or t(16;16)(p13.1;q22); CBFB-MYH11
Acute myeloid leukaemia promyelocytic leukaemia with t(15;17)(q22;q12); PML-RARA
Acute myeloid leukaemia with t(9;11)(p22;q23); MLLT3-MLL
Acute myeloid leukaemia with t(6;9)(p23;q34); DEK-NUP214
Acute myeloid leukaemia with inv(3)(q21q26.2) or t(3;3)(q21;q26.2); RPN1-EVI1
Acute myeloid leukaemia with mutated NPM1*
Acute myeloid leukaemia with mutated CEBPA*
Acute myeloid leukaemia with myelodysplasia-related changes
Therapy-related myeloid neoplasia
Myeloid sarcoma
Myeloid proliferations related to Down's syndrome
Transient abnormal myelopoiesis
Myeloid leukaemia associated with Down's syndrome
Blastic plasmacytoid dendritic cell neoplasm

*Provisional entities.

(<1%) and they can only be demonstrated in 60-80% of patients. These AML-CFUs form small colonies of 20-100 cells *in vitro* and are exclusively dependent on the presence of colony-stimulating factors (CSFs) for growth.[11]

Russell et al showed that the growth and survival of cancer cells is frequently driven by the same factors that stimulate normal cell proliferation.[12] What is even more conclusive is the fact that the haematopoietic microenvironment regulates normal and abnormal haematopoiesis via cell-cell interactions and that it generates specific hormones and cytokines: erythropoietin, interleukin 3 (IL-3), granulocyte-monocyte colony-stimulating factor (GM-CSF), monocyte-macrophage colony-stimulating factor (M-CSF), granulocyte colony-stimulating factor (G-CSF), interleukin 5, interleukin 4, and other less well-defined factors. The experimental proof for this assumption can be found in Metcalf's recent work on colony-stimulating factors.[13]

McCulloch et al, previously showed that several cytokines exert a synergistic effect on blast clonogenic cells when used in conjunction with IL-3 or GM-CSF: IL-1, IL-6, TNFa and IFN-y.[14] The response of AML blasts to these cytokines is, however, heterogeneous, for example some samples respond to IL-1 but not to IL-6, and vice versa. Furthermore, colony formation is dependent on the cell concentration, despite the presence of a combination of synergistic growth factors. In contrast, Caceres-Cortes et al demonstrated instances of a homogeneous response to the synergistic effect of Stem Cell Factor (SCF) in almost all AML samples. The addition of SCF significantly reduced the requirement of GM-CSF, as well as in cellular interactions, for blast colony formation. Furthermore, immunofluorescence studies revealed the presence of membrane-bound SCF (mSCF) in AML blasts.[15]

This work indicated that colony formation as a function of cell concentration was better described by a model with two limiting parameters: the colony-forming cell itself and an «interacting» cell. Interestingly, the addition of high concentrations of soluble SCF was sufficient to alleviate the requirement for cellular interactions. These observations led the authors to investigate the possibility that membrane SCF could participate in the process of cell-cell interactions. It was established that both undifferentiated (FAB M1, $CD34^+$) and differentiating (FAB M4/5 and $CD13^+$) AML blasts express mSCF (Figure 1).[16]

Using an antisense strategy with phosphorothioate-modified oligonucleotides that specifically target SCF without affecting other surface markers, the authors provided direct evidence of a role of mSCF and c-Kit in cellular interactions and cell survival.[16] Figure 1 illustrates the expression of surface marker CD34 in AML stem cells. As the cells differentiate, they acquire myeloid surface markers such as CD13 and lose CD34 expression. Because mSCF was not detected in all of the cells, the authors addressed the

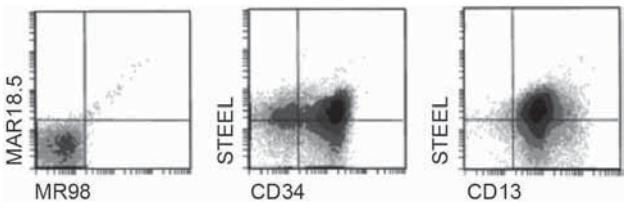


Figure 1. Both $CD34^+$ and $CD13^+$ cells express SCF. Primary AML blasts (AML 32) were stained with CD34 or CD13 (IgG1) directly conjugated to FITC and the antibody against SCF (7H6, IgG2a) followed by a goat antimouse IgG2a coupled to PE. Control cells were stained with isotype-matched irrelevant antibodies (MAR18.5, IgG2a and MR98, IgG1). The data are representative of two independent labelling studies with AML 32 blasts. (Taken from Caceres-Cortes et al., Cancer Res. 2001;61:6281-9).

question of whether mSCF expression was a property of leukaemic progenitors and/or of differentiating leukaemic cells. Double labelling indicated that both $CD34^+$ and $CD34^-$ AML blasts expressed mSCF. Furthermore, most $mSCF^+$ cells were also $CD13^+$. Thus, mSCF was expressed by leukaemic progenitors and differentiating leukaemic cells, consistent with the fact that the expression of mSCF was not restricted to a FAB subtype. The blast cells of AML express c-Kit and respond to SCF in culture.[17] Important supplementary evidence was forthcoming in Hassan's interesting observations on the effect of SCF, where it interacts with other cytokines to preserve the viability of haematopoietic stem and progenitor cells in order to influence their entry into the cell cycle and to facilitate their proliferation and differentiation.[18] Co-expression of Kit with its respective ligand implies that this receptor might contribute to leukaemogenesis in some patients with AML through autocrine, paracrine or intracrine interactive stimulation.[19]

It is evident from the above that blast cell proliferation takes place in the environment of circuits, in which cells produce their own growth factors and use them to proliferate.

Inhibitors of c-Kit

There are more than 200 chromosomal translocations and several point mutations in AML, which makes it impossible to examine them one by one as therapeutic targets. Functionally, oncogenes that induce development of the leukaemic phenotype can be classified into two groups: genes that increase cell proliferation and those that block the normal process of cellular differentiation.[20]

Among the oncogenes that promote cell growth we find mutations in the intracellular signalling protein Ras (20% of patients) and the activation of tyrosine kinases (40% of patients), such as growth factor receptor tyrosine kinases or receptor-associated intracellular kinases. Among the oncogenes that intervene in cell differentiation, many transcription factors are important for normal myeloid development.

The receptor tyrosine kinase (RTK) c-Kit has a pivotal role in melanogenesis, gametogenesis and haematopoiesis.[21] It is expressed by myeloblasts in about 60% to 80% of patients, and the most frequently observed activating RTK mutations in AML are mutations or internal tandem duplications in c-Kit, with an overall incidence of 17%.[21] The identification of a small-molecule tyrosine kinase inhibitor (Tyrphostin B42 or AG490) capable of blocking c-Kit at a concentration of 3 μM introduces a change in the perspective regarding the treatment of AML, even if AG490 is synthesized as a selective inhibitor of EGFR without the normal detriments of haematopoiesis.[22,23] The c-Kit inhibitor AG-490 was found to induce both apoptosis and autophagy *in vitro*, and the inhibition of autophagy significantly augmented the cytotoxic effects of AG-490 in AML.[24] Data suggest that new Tyrphostins could be modelled on AG490 for increased potency and selectivity towards c-Kit. Table 2 shows recently registered compounds that exhibit increased efficacy by neutralizing c-Kit. Most of the current kinase inhibitors target the ATP-binding site of the kinase catalytic domain, although they show marked chemical structural differences between them. Although the gain-of-function mutation in c-Kit is a common event in AML, the findings indicate that multiple genes contribute to the induction of leukaemia and that no single genetic abnormality is sufficient in itself to induce disease. This assumption has important consequences for therapy, as it provides a motive for the exploration of combined treatment modalities that simultaneously target multiple pathways in the leukaemic cell, including c-Kit as one of the most important ones.

Cancer stem cells in AML

The cancer stem cell hypothesis has gained considerable importance in recent years.[33] This theory states that cells in a tumour are organized in a hierarchy similar to that of normal tissues and are maintained by a small subset of tumour cells that are ultimately responsible for tumour formation and growth. These cells, defined as "cancer stem cells" (CSCs) or "tumour initiating cells" (TICs),[34] possess several key properties of normal tissue stem cells including self-renewal, unlimited proliferative potential, infrequent or slow replication, resistance to toxic drugs and high DNA repair capacity. However, unlike highly regulated tissue stem cells, CSCs demonstrate deregulated self-renewal/differentiation programmes and produce daughter cells (absolute majority) that arrest at various stages of differentiation. The bulk of the tumour is characterized by rapid replication, limited proliferative potential and the inability to form a new tumour. Only CSCs are able to initiate tumour formation as they are exclusively capable

Table 2. Recently described inhibitors of c-Kit (Continued on page prox)

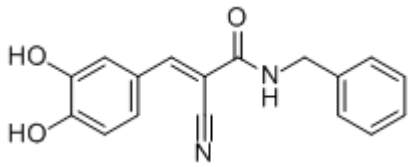
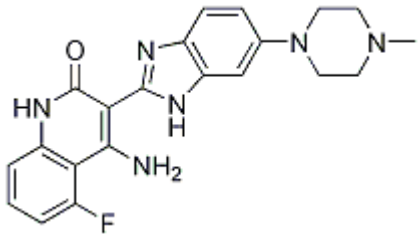
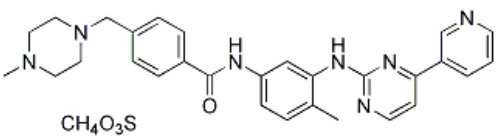
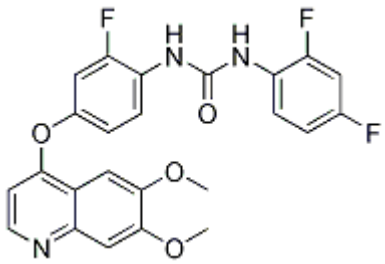
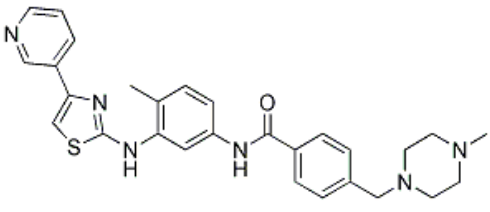
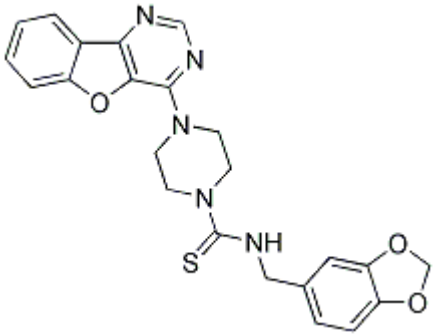
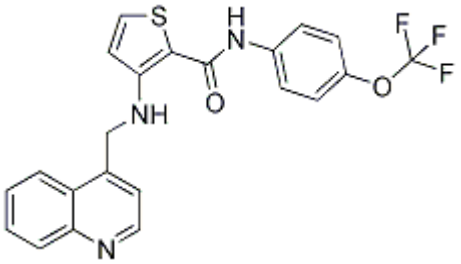
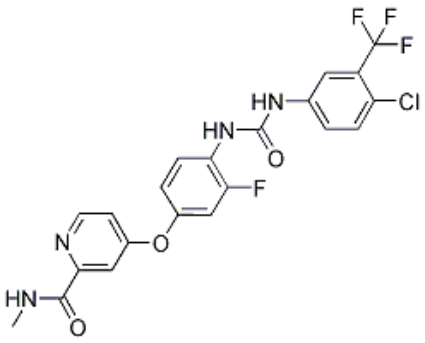
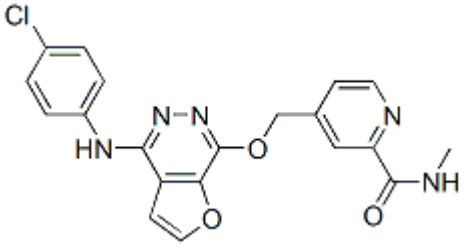
Compound	*Half inhibitory concentration (IC(50))*	*Chemical structure*	*Ref.*
AG-490 (synonym Tyrphostin B42)	IC50 c-Kit 3 μM		15
Dovitinib (synonyms TKI258, CHIR-258)	IC50 c-Kit 2 nM		25
Imatinib Mesylate (synonyms Gleevec, Glivec, CGP-57148B, STI-571)	IC50 c-Kit 100 nM		26
Ki8751	IC50 c-Kit 40 nM		27
Masitinib (synonyms AB1010, Masivet)	IC50 c-Kit 200 ± 40 nM.		28

Table 2. Recently described inhibitors of c-Kit (Continued)

Compound	*Half inhibitory concentration (IC(50))*	*Chemical structure*	*Ref.*
MP-470	IC50 c-Kit 1 µM.		29
OSI-930	IC50 c-Kit 100 nM		30
Regorafenib (synonyms BAY 73-4506)	IC50 c-Kit 17 nM		31
Telatinib (synonyms BAY 57-9352)	IC50 c-Kit 19 nM		32

of self-renewal.[34] In AML, many of the remaining cells with a normal appearance exhibit various abnormalities. One interpretation is that these cells are descendants of leukaemic cells that have succeeded in overcoming the differentiation block that exists in AML. Killmann[35] drew attention to a single circumstance, one that might be regarded from the biologist's point of view as an abnormality, in the evolution of leukaemia. He observed evidence of red cell precursors of leukaemic descent and claimed that the target cell of AML was the stem cell. Occasionally in AML, too many stem cells also develop into abnormal red blood cells or platelets.

While the literature deals admirably and abundantly with the pathology, symptoms and treatments in AML, there are a considerable number of cases that present anatomical features and technical difficulties that require consideration from a fundamental research standpoint. Until now, the accurate identification and separation of minimal residual cells (the alleged cancer stem cells) have proven futile due to a lack of identifiable attributes for singling out these cells from the heterogeneous tumour bulk. Markers that reliably identify cancer stem cells in AML could assist in the prognosis and could improve strategies for therapy. Clear evidence for the CSC theory comes from studies by Dick and colleagues, who demonstrated some years ago that only rare cells in AML were able to initiate leukaemia in murine models, and serial transplantation studies revealed that these cells have a high self-renewal capacity.[36,37] The cell responsible for tumour initiation was identified as having a CD34+ CD38- phenotype, which was particularly interesting as bulk AML samples tend to be CD34-. Furthermore, CD34+ CD38- is a phenotype characteristic of normal haematopoietic stem cells (HSC), indicating that putative CSCs might have a primitive phenotype. Bonnet et al. [38] found that as few as 5×10^3 CD34+ CD38- cells could engraft an immunecompromised mouse, while 100 times more CD34- or CD34+ CD38+ cells from the same donor could not. Importantly, the tumours derived from injecting the CD34+ CD38- cells were heterogeneous and composed of a mixture of tumourigenic and non-tumourigenic cells similar to the donor sample. Since then, stem-like cells have been identified in a variety of human malignancies, including other leukaemias and solid tumours such as breast, colon, brain, head and neck, lung, pancreatic and nasopharyngeal cancers and melanomas [39]. Remarkably, even established cancer cell lines that have been grown *in vitro* for many years appear to contain CSC-like populations that can be isolated and which are highly tumourigenic.[40]

Autophagy, the degradation of cytoplasmic components, is an evolutionarily conserved homeostatic process involved in environmental adaptation, lifespan determination and tumour development.[41] Recent evidence in mice suggests that autophagic defects in haematopoietic stem cells are implicated in leukaemia. Indeed, mice lacking Atg7 in haematopoietic stem cells develop an atypical myeloproliferation resembling human myelodysplastic syndrome, progressing to AML. Studies suggest that the accumulation of damaged mitochondria results in cell transformation.[42] Interestingly, bone marrow cells from preleukaemic patients are characterized by mitochondrial abnormalities and increased rates of cell death. A role of autophagy in the transformation into cancer has been proposed in other cancer types.[24,42] The mammalian target of rapamycin (mTOR) serine/threonine kinase, an inhibitor of autophagy, is often constitutively activated in AML.[43] Furthermore, Chiacchiera and Simone reported that autophagy or self-cannibalization is a pro-survival mechanism for cancer cells that emerge under conditions imposed by nutrients or growth factor deprivation.[44] The cytoprotective role of autophagy following chemotherapy has been confirmed by other investigators.[45,46,47]

Autophagy and apoptosis can be triggered by common upstream signals, and sometimes this result in combined autophagy and apoptosis; but this could also be explained by how cells divide. As in many tissues, rare populations of cancer stem cells have been characterized in AML. The ability of stem cells to undergo both asymmetric (self-renewal) and symmetric (division to produce a more differentiated cells) cell division is what defines them as stem cells. Understanding the mechanisms of molecular genetics that govern the self-renewal and proliferation of these cells will be important in clarifying why cancer stem cells persist by autophagy whereas daughter cells are prone to suffering apoptosis. Symmetric cell division is defined as the generation of daughter cells destined to acquire the same fate, whereas stem cells divide asymmetrically to generate one daughter with a stem-cell fate and one daughter with a different fate. The normal differentiation of progenitor cells into lineage-specific cells results in a loss of multi-lineage potential and follows a hierarchical pattern.[48]

If malignant stem cells behave similarly by dividing asymmetrically then they could have evolved from normal stem cells. Theoretically, if we consider, in a very simple manner, a solid tumour composed of a heterogeneous cell population due to the presence of stem cells with autophagic properties and other cells with apoptosis properties, we might have a different number of possibilities of arranging the cells into the tumour, named permutations (P) or approximate entropy (by definition S=Ω!k):

$$P = \Omega!/\alpha!\beta!$$

where:

Ω! (omega factorial) = the number of possibilities of arranging populations 1 + 2

α! (alfa factorial) = the number of arrays of cell population 1

β! (beta factorial) = the number of arrays of cell population 2

In tumours composed of cells with the same potential to create a new tumour, α!! tends to be 1, and in tumours with different components (tumour stem cells and other components that do not have this potential) it is understood that the arrangement or entropy would be different, which should be taken into account when considering metastases.[49] One can typically infer the existence of a cancer stem cell within a tumour when it has more entropy n-permutations, and then interpret it as a more "aggressive" or metastatic compared to a tumour consisting of two or more cell types with less entropy. We are uncertain whether or not these variations in the behaviour of the cells during degeneration are dependent upon a difference in the type of the leukaemic cell or upon differences in the individual cells themselves. In particular, we have observed a side population of cells in autophagy while the rest of the cell population dies by apoptosis under AG490 treatment in the TF-1 cell line.[50] Inhibition of c-Kit signaling induced combinations of cell death: autophagy (compensatory mechanism toward survival) and apoptosis. Usually, tumour cells activate autophagy in response to metabolic demands related to rapid cell proliferation. As shown in preclinical models, inhibition of autophagy restored chemosensitivity and enhanced tumour cell death.

Concluding remarks

This brief review has presented the bias that growth factor responsiveness is a unique biological characteristic of AML blasts with an almost homogeneous response to the synergistic effect of SCF. In short, it is alleged that AML blasts are comprised of differentiated cells that have a limited life-span, and cancer stem cells/tumour initiating cells, which are undifferentiated cells with an unlimited replication capacity and the ability to differentiate. In estimating the value of this distinction, everything depends upon the significance of stratification. The cancer stem cell hypothesis suggests that if cancer stem cells are selectively ablated, tumour growth will cease and eventually the tumour will regress. Under these circumstances, the precise nature of the heterogeneity within neoplasms is important for understanding cancer progression, cancer stem cells and therapeutic resistance. The number of new, active agents is rapidly increasing and personalized medicine has definitively arrived. Treatment choices are starting to be made upon tumour characteristics, and more effective and tolerable agents are now available. Protein kinases are now one of the most intensely pursued classes of drug targets. Over 30 distinct kinase targets and over 200 kinase inhibitors are in clinical development for the treatment of cancer. In our experience, we have observed instances where the c-Kit inhibitor AG-490 induced both apoptosis and autophagy *in vitro*, and that the inhibition of autophagy could significantly augment the cytotoxic effect of AG-490 in AML. A detailed characterization of stem cell subcomponents, characterized by autophagy behaviour, is essential as it may help to clarify their functions in cancer biology.

Competing interests

This work was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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