CLINICAL INVESTIGATION

Cancer stem cells as a therapeutic target of the future

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Cancer stem cells represent one of the dominant themes of cancer research today. Although not accepted by all and without reservation, and probably not valid for all tumors, the evidence for viewing tumors as a sort of aberrant tissue with intrinsic hierarchical organization is mounting. Cancer stem cells are placed at the top of this hierarchy, making them responsible for continuous tumor growth and progression. At the same time, they have several properties that make them difficult to eradicate by conventional therapeutic approaches. First, they are, in many tumor types at least, rather quiescent, providing them with little 'natural' sensitivity to classical cancer chemotherapy. More importantly and aside from this rather passive defense mechanism, cancer stem cells have been reported to dispose of various active mechanisms of chemo- and radio-resistance. Cancer stem cells constitutively express several efflux pumps of the ATP Binding Cassette (ABC) family (ABCB1 – MDR1; ABCC1 – MRP1; ABCG2 – BCRP) and are thus able to actively get rid of many anticancer drugs. Another relatively universal stem cell protein – aldehyde dehydrogenase – can enzymatically inactivate certain chemotherapeutics, such as cyclophosphamide. Treating cancer by inducing DNA damage to cancer cells, whether by chemotherapy (e.g., platinum compounds) or radiotherapy, requires the cooperation of endogenous reactive oxygen radicals, the levels of which seem to be distinctly low in all stem cells. In addition, cancer stem cells show an increased DNA-repair activity and decreased apoptotic competence and, consequently, even if a cancer stem cell incurs DNA damage, it can cope with it. Together, all these active and passive defense mechanisms, collectively summoned up as self-protection, make cancer stem cells resistant to traditional anticancer therapy. Even in the case of a complete clinical response, they often survive and initiate later tumor recurrence; they are, in fact selected for by current cancer therapies [1,2].

Interestingly, many if not all of the above cited mechanisms of resistance become widespread in terminal cancer stages. The transition between the initially sensitive tumor with a small resistant cancer stem cell subpopulation and a completely resistant tumor is poorly understood. Sometimes, the expression 'exquisity' of cancer stem cells might become fixed in the entire cancer cell population by a robust genetic change (e.g., a constitutive MDR1 overexpression as a consequence of gene amplification or chromosomal translocation). Another mechanism might be a loss of the hierarchical structure as a consequence of the loss of the differentiation capacity of cancer stem cells. Then, the therapy sensitive differentiated tumor cells simply disappear and the stem cell phenotype with all the intrinsic resistance mechanisms becomes predominant in the cancer cell population. In any case, it is interesting that, although all of the above cited mechanisms of active resistance are shared between normal and cancer stem cells, this transition to a universal pan-cell resistance could never be observed in normal tissues. It has, for example, never been observed that repeated cycles of chemotherapy, each producing a temporary depression of hematopoiesis, would lead to the appearance of a che-

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moresistant hematopoietic system. Apparently, a degree of genomic instability is necessary to promote this step in the acquisition of generalized therapy resistance in cancer cells. Thus, even if the hierarchical principle of organization is retained, tumor development and progression also involve aspects of clonal evolution. And the quarrel between the advocates of the hierarchical stem cell model and those who prefer the stochastic clonal evolution model of cancer remains unsolved [3,4].

One way to settle this argument between the adherents and opponents of the cancer stem cell concept would be to find a cancer stem cell-specific drug. If active clinically, or at least in a realistic experimental model, then even the most stubborn sceptics would have to acknowledge that there could be something right about this theory. And, indeed, we now witness an interesting new development in the field. A traditional rational approach to cancer stem cell-specific therapy would be to identify a specific signaling pathway crucial for cancer stem cell self-renewal or viability and to block it with a specific inhibitor. Several such crucial stemness signaling pathways have been identified (e.g., Wnt/β-catenin, Hedgehog, Notch, Hippo) and several specific inhibitors are now in early phases of clinical testing [5]. These efforts are facing two basic problems. First, a variable (but often quite large) part of the stemness signaling circuitry is shared between normal and cancer stem cells [6]. These pathway-specific inhibitors thus carry the danger of toxicity towards normal stem cells, and finding their optimal place in the anticancer therapeutic armamentarium might not be easy. Second, the stemness pathway-specific inhibitors are, most frequently, new drugs and their development, through all the required phases of clinical testing, is by necessity long and expensive.

Nevertheless, another unexpected avenue of development in the search for cancer stem cell-targeted therapeutics appeared recently. Surprisingly, it turned out that some old drugs that have been in routine use in human or veterinary medicine for decades, most often for indications other than cancer, could be very effective at targeting cancer stem cells. Identifying such a new application for an old drug would obviously carry tremendous advantages – these drugs are frequently no longer under patent protection; that is, they are much cheaper than the newly introduced drugs, and their pharmacokinetics and side effects are well characterized. Hot candidates among these old chaps are disulfiram, prescribed for the treatment of alcoholism, several antipsychotic drugs, such as thioridazine or trifluoperazine, the antimalarial mefloquine, as well as the veterinary antibiotic salinomycin.

The biological logic behind their efficacy in stem cell targeting is only beginning to emerge. Disulfiram's use in treating alcoholism is based on its irreversible inhibition of aldehyde dehydrogenase, a key enzyme in alcohol metabolism [7]. As specific subtypes of aldehyde dehydrogenase are also a part of stem cell self-protection (see above), its stem cell-specific targeting properties follow quite naturally. Likewise, the stem cell-specific targeting of thioridazine and trifluoperazine could be explained by the recent finding that cancer stem cells (and, most intriguingly, not the corresponding normal stem cells) express dopamine receptors – both these drugs act as specific dopamine receptor antagonists, and, as such, are traditionally used to treat psychiatric disorders like schizophrenia [8,9]. Nevertheless, it is gradually turning out that some of these old drugs can exert rather complex effects on cancer stem cells. Disulfiram has thus been reported to act as an efficacious inhibitor of polo-like kinase 1 and O6-methylguanine methyltransferase (MGMT) [10], both key enzymes of glioblastoma stem cell biology, and of the proteasome [11]. This proteasome inhibition results in the downmodulation of the transcription factor NFκB, the overactivity of which has been described in many normal and cancer (stem) cells [12]. Likewise, salinomycin, an extensively used anticoccidial veterinary drug, has been reported to be an inhibitor of the ABCB1 efflux pump, the Wnt/β-catenine signaling pathway and of oxidative phosphorylation in mitochondria, as well as to act as a specific potassium ionophore [13,14]. Mefloquine primarily works via lysosme disruption and the consequent release of reactive oxygen species and lysosomal proteases; albeit, this effect is not cancer stem cell-specific, impairing the entire cancer cell population, cancer stem cells are co-targeted as well, at least in acute myeloid leukemia [15].

Given that pan-resistant tumors might co-opt resistance mechanisms of cancer stem cells, it comes as little surprise that these new stem cell targeting drugs also show promising effects on chemoresistant cancer cell lines. Again, these effects often show a biological logic. O6-methylguanine methyltransferase overexpression is a general mechanism, by which glioblastomas avoid the genotoxic effect of temozolomide, and inhibition of this DNA repair enzyme by disulfiram leads, accordingly, to the chemosensitization of temozolomide-resistant glioblastoma cell lines [10,16]. At the same time, the disulfiram-mediated inhibition of NFκB should lead to a drop in expression of many antiapoptotic genes, again sensitizing the cells to the effects of many anticancer drugs [12,17]; its inhibition of aldehyde dehydrogenase that enzymatically inactivates certain anticancer drugs has already been mentioned. A slightly different mechanism seems to be operating in dopamine receptor antagonists – they seem to primarily induce a differentiation (i.e., the loss

of stemness) of cancer stem cells, leading to a loss of all their self-protection mechanisms and, correspondingly, increasing their sensitivity to conventional anticancer drugs [8].

It seems, therefore, that the most rational application of these discoveries lies in the integration of these new (at least within the context of oncology) drugs into combination chemotherapy regimens. However, another aspect of cancer stem cell biology argues in favor of this conclusion. One of the hallmarks of cancer stem cells and at the same time a crucial difference between cancer and normal stem cells is the plasticity of the former [18,19]. The cancer stem cell population is in many tumors in a constant change, diminishing via differentiation of cancer stem cells and at the same time being continuously replenished by de-differentiation processes. Single-shot eradication of cancer stem cells would thus, contrary to earlier expectations, most probably fail to lead to a cancer cure [20]. Simultaneous targeting of stem and differentiated cancer cell populations could, thus, represent the long awaited strategy for attacking cancer in a way that it would not be able to easily defend against. Given the fact that some of these new drugs are in fact old ones, we can only hope that this paradigmatic shift in cancer treatment will be soon be coming into clinical practice.

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