Reviewer's report

Title: Acquisition of Anoikis Resistance in Human Osteosarcoma Cells Does Not Alter Sensitivity to Chemotherapeutic Agents

Version: 1 Date: 11 October 2004

Reviewer: Janusz Rak

Reviewer's report:

General
This is an interesting paper, which provides evidence to suggest that in the case of human osteosarcoma cell line (SAOS-2) the susceptibility to cell death induced by detachment (anoikis) can be separated from apoptosis brought about by exposure of these cells to several anticancer drugs. It is therefore argued that both types of cell death are likely executed by different mechanisms, and hence resistance to one may not be predictive of the other.

While there is some unquestionable value in this analysis, the underlying assumption as to the commonality of cell survival regulating mechanisms under a variety of circumstances in cancer is somewhat contrived. This is for a number of reasons: (i) The literature does contain strong indications as to an overlap between anoikis-regulating processes (adhesion signaling) and changes in sensitivity to anticancer agents, but is far from equating these two events (see Schmitt et al, Cell, 2002), (ii) It is obvious that certain types of tumors (e.g. hematopoietic malignancies) often remain anoikis-resistant (a priori) and at the same time are highly responsive to chemotherapy. It is noteworthy however, that adhesion can modulate the latter property (e.g. see Matsunaga et al Nat.Med 2003), (iii) Altered expression of survival genes (due to inductive and/or selective events) as a result of prolonged exposure to chemotherapy is hardly inconsequential for the degree of anoikis resistance, but this does not necessarily mean that the reverse is also true; (iv) Anoikis resistance could, at least in some cases (SAOS-2?) be executed via mechanisms related to altered adhesion signaling (to which apoptotic consequences may be secondary), and this may or may not affect responses to genotoxic insults. Apart from these conceptual (and admittedly debatable) issues, the paper comes across as very preliminary, phenomenological and descriptive in nature.

Major Compulsory Revisions (that the author must respond to before a decision on publication can be reached)

The authors should strengthen their study in the following manner:

1. Therapeutic implications of various relationships between anoikis- and anticancer drug resistance of cancer cells in vitro must ultimately pass the test in vivo, i.e. in the context of an overt tumor growth. The authors should examine whether SAOSp and SAOSar cell lines possess similar or different tumorigenic capacities and whether their responsiveness to anticancer agents is similar or different in vivo.

2. In a qualitative sense, sensitivity to anoikis akin to that the authors describe for SAOS-2p cells could be viewed as unusual among cancer cells, the well-known hallmark of which is the ability to grow three-dimensionally in semisolid media, or as spheroids (i.e. in the anoikis-resistant manner). It could be argued, however, that in a qualitative sense some cancer cells may be more resistant to anoikis than others, and this would constitute the only ground to accept the basic premises of the present study. If so, SAOS-2-unrelated cell lines (osteosarcoma or otherwise) should be included in the analysis to determine how common the separation between chemo-sensitivity and
anoikis-sensitivity really is, and whether SAOS-2 cells represent a trend or a peculiarity in this regard.

3. More meticulous and mechanistic analysis could considerably strengthen the paper. For instance, longer time courses (for anoikis induction and drug treatment) would provide insights as to the kinetics and/or magnitude of cell death under the various conditions. The paper would also benefit from inclusion of more specific/sensitive apoptosis assays (e.g. TUNEL, Annexin V staining, Cell death ELISA), biochemical evidence for (differential?) caspase activation, as well as evidence that caspase inhibitors (e.g. zVAD peptides) block either or both forms of cell death (i.e. induced by drugs or suspension culture). Also simple analysis of key members of the bcl-2 family, Fas/FasL and other effectors could offer valuable insights. All these experiments are relatively simple and appropriate reagents are readily available from commercial sources.

---------------------------------------------------------------------------------------------------------------------

Minor Essential Revisions (such as missing labels on figures, or the wrong use of a term, which the author can be trusted to correct)

1. The assay described on page 7 is based on the cell membrane damage and hence not suitable to specifically detect apoptosis.
2. The primary mechanism of cell death in the presence of various chemotherapeutics can vary from mitotic catastrophe, senescence and bona fide apoptosis induction. This and other intricacies are not fully reflected in Discussion, which should be more focused on the authors' main finding, i.e. anoikis resistance without an apparent apoptosis resistance.

---------------------------------------------------------------------------------------------------------------------

Discretionary Revisions (which the author can choose to ignore)

1. Many of the cited references on anoikis, anoikis resistance, chemotherapy resistance and other aspects are review papers and secondary reports rather than seminal work. Also, several recent important and relevant papers in these areas have been omitted in the paper e.g. studies by Douma et al Nature, 2004, Matsunaga et al, Nat. Med. 2003, Schmitt et al Cell 2002. The authors may consider re-thinking this aspect of their paper.

What next?: Unable to decide on acceptance or rejection until the authors have responded to the major compulsory revisions

Level of interest: An article whose findings are important to those with closely related research interests

Quality of written English: Acceptable

Statistical review: No

Declaration of competing interests: None