Reviewer's report

Title: Effects of alpha fetoprotein on escape of Bel 7402 cells from attack of lymphocytes

Version: 1 Date: 10 May 2005

Reviewer: Gerald J Mizejewski

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General
The authors have shown that HAFP was able to suppress or disable the growth of Jurkat (T-Lymocyte cells) while enhancing the proliferation of hepatoma (Bel) cells. This dual effect in co-culture would effectively allow tumor cells to escape immune elimination and to flourish in conditions of tumor growth and progression. They showed that Fas expression was enhanced in hepatoma cells co-cultured with T-lymphocytes and that HAFP could block that stimulation in the T-lymphocytes. In comparison, the expression of FasL in the hepatoma cells was enhanced as was overexpression of Fas on the surface of the T-lymphocytes (both HAFP-induced). These latter observations were remarkably significant in that the accelerated death of the lymphocyte (via Fas-to-FasL interaction) would allow the hepatoma cells to proliferate unabated, and thus escape immune elimination. The results were even more impressive when viewed in the light of caspase-3 protein expression (via Western blots). Caspase-3 expression was clearly abrogated in the hepatoma cells, while visually enhanced in the T-cells. Although AFP did not affect survivin protein levels, the authors did report a slight increase of the protein in the co-cultured Jurkat cells. Survivin gene signatures have been reported to predict aggressive growth in tumors (Can. Res 65:3531, 2005).

The authors should include in their paper (in a final version) a recent report published in J. Immunol. 173:1772, 2004. This report serves to confirm and extend the present authors’ contention that AFP aids hepatoma cells in escaping immune elimination by the induction of apoptosis, dysfunction, and functional impairment of immune dendritic cells (DC) in hepatoma – bearing patients. Dendritic cells are one of the most potent antigen-processing monocyctic cells during the initiation of immune responses against pathogens and tumors cells. The authors of this 2004 report detected a down regulation of surface molecules (as in the present report) as well as an inhibition of the T-cell stimulatory capacity. The reported that HAFP reduced the ability of the dendritic cells to produce TNF-? and IL-12 and to induce cell death of the monocyte-derived DCs.

AFP is known to regulate growth by a mechanism that includes apoptosis regulation (Exp. Biol. Med. 226:377, 2001). Studies have revealed that HAFP can induce apoptosis in various tumor cell culture lines including hepatomas and lymphblastomas (Tumor Biol. 18: 30, 1997) but can be eliminated by cell exposure to IL-2 (Eur. Cytokine Network 9: 448, 1998). The activation of caspase-3 by AFP was first demonstrated Dudich et al (Biochem 38: 10406, 1999) and was found to be independent from caspase-1, 8, and 9. The cytotoxic effect of AFP and apoptosis are related and do not have to be linked to Bcl-2, Bcl-L, and the TNF receptor cascade. Thus, it has been established that AFP can induce apoptosis in tumor cells via a pathway independent of CD95 (FAS) and TNFR1 and TNFR2 by activation of the caspase-3 proteases (Europ. J. Biochem 255: 750, 1999). Finally, Dudich’s groups has further provided evidence that AFP regulates, in a positive fashion, cytochrome-C-mediated caspase activation and apoptosome protein complex formation (Europ. J. Biochem 270: 4388, 2003).

The practice of co-culture experiments has recently gained investigational prominence in the field of biomedical research. By co-incubating immunological-associated cells (lymphocytes) with tumor cells in defined culture media, the mechanism of tumor cells escaping immune elimination has come to the forefront. Co-culture experiments have been successfully employed to study the relationship of apoptotic interactions among tumor cells versus lymphocytes (Clin. Cancer Res. 5: 1219, 1999; J.
The authors of the present report have effectively employed this technological method to advance the knowledge of how AFP influences the lymphocyte-to-hepatoma cell relationship regarding the Fas (CD95) and FasL (ligand) interaction. By expressing FasL on their cell surface, tumor cells are able to present lymphocytes with a binding target for the lymphocyte Fas-expressed proteins. Apparently, the molecular complex formed between lymphocyte-bound Fas and hepatoma-expressed FasL is sufficient to impose a cell death sentence upon the lymphocyte. With the lymphocytes destroyed or neutralized, the hepatomas are free to grow and proliferate.

Abstract: line-8 “whereas, induce the FasL expression”
line-13: “mono-antibody” should read monoclonal antibody.

Background: Page 3, line 9: The sentence starting “Recently, some studies on the mechanisms” is very long and convoluted sentence, and requires grammatical editing.

Results: Page 6, line 3: The sentence ending in the phrase “even though no AFP treatment” needs revision.

Results: Page 6, line 9: At the end of the paragraph, the sentence ending in “a slight increment in co-cultured Jurket cells”, needs a closing verb.

Discussion: page-6, 1st paragraph, line-7: Sentence stating “Hereby, the implication was emerged” should read “has emerged”.

Discussion: Page 7, line 19: The “term specific membrane AFP receptors” cannot be used as is and must be further qualified. It is apparent that the authors did not produce any results that demonstrated the presence of any type of “AFP receptor”. The AFP receptor for apoptosis has not been elucidated to date. Several authors have attempted to characterize a cell surface AFP receptor but each report yields differing and confusing results (i.e. J. Clin. Invest. 90: 1530, 1992; PNAS 82: 3301, 1985; Tum. Biol 14: 116, 1993; BBRC 122: 1322, 1984).

References: Page 9; #12 should read “Mizejewski GJ, and MacColl, R.” as authors.

What next?: Accept after minor essential revisions

Level of interest: An article of importance in its field

Quality of written English: Needs some language corrections before being published

Statistical review: No

Declaration of competing interests:

I declare that I have no competing interests.