Biomarkers, November 2005; 10(Supplement 1): S69–S76

Taylor & Francis
Taylor & Francis Group

Can we develop biomarkers that predict response of cancer patients to immunotherapy?

B. BONAVIDA[1], S. HUERTA-YEPEZ[1], L. GOODGLICK[2], Y. MIZUTANI[3], & T. MIKI[3]

[1]*Department of Microbiology, Immunology and Molecular Genetics,* [2]*Department of Pathology and Laboratory Medicine, David Geffen Medical School, University of California at Los Angeles, Los Angeles, CA, USA,* [3]*Department of Urology at the Kyoto Prefectural, University of Medicine in Kyoto, Japan*

Abstract

Primary objective: The primary objective is to delineate the potential utility of cancer biomarkers that correlate and predict response to immunotherapy in cancer patients who are refractory to conventional therapeutics. Unlike significant development of biomarkers that predict response to chemotherapy, very few biomarkers have been developed to predict the response to immunotherapy.

Main outcomes and results: This article describes briefly the importance of characterizing and validating biomarkers for immunotherapy. A few examples have been provided, such as the transcription factor NF-κB, the transcription repressor Yin-Yang 1 (YY1), the pro-apoptotic gene product (Smac/DIABLO) and the circulating Fas and Fas ligand. These biomarkers have been determined to be of prognostic significance in different cancers.

Conclusions: Immunotherapy is considered as an alternative therapy in the treatment of cancer patients who are refractory to chemotherapy/radiation/hormonal therapies. Cross-resistance to apoptosis develops between cancer cells that are resistant to conventional therapeutics and immunotherapy. Therefore, it is important to develop biomarkers that will determine patient response to immunotherapy.

Keywords: *Immunotherapy, YY1, Fas, Smac/DIABLO, NF-κB, biomarkers*

Introduction

Significant advances have been made in the treatment of cancer by chemotherapeutic drugs, hormonal drugs and radiation. However, the development and/or acquisition of tumour resistance to such treatments present a major drawback (Patel & Rothenberg 1994). While patients with early and localized tumours respond to standard therapy, the majority of cancer patients afflicted with advanced metastatic tumours are unresponsive to further treatments and these patients will eventually succumb to incurable disease and die. The mechanism of drug resistance is complex and multi-factorial (reviewed by Pommier et al. 2004). Much of the research efforts today are focused on searching for alternative therapeutic strategies that are aimed to reverse or

Correspondence: Benjamin Bonavida, PhD, Department of Microbiology, Immunology and Molecular Genetics, CHS A2-060, 10833 Le Conte Ave., Los Angeles, CA 90095, USA. Email: bbonavida@mednet.ucla.edu

ISSN 1354-750X print/ISSN 1366-5804 online
DOI: 10.1080/13547500500216827

bypass drug-related resistance mechanisms (Tan et al. 2000). Tumour immunotherapy is an ideal therapeutic approach because it offers several advantages over chemo/hormonal/radio-therapies including low organ toxicity and high tumour selectivity. In immunotherapy, the tumour killing agents are derived from the host's own immune system.

Immunotherapeutic strategies under investigation consider that chemo-resistant tumours are sensitive to immunotherapy. It has been assumed that immunotherapy attacks tumour cells using different mechanisms of action and may not be subjected to the drug-resistant mechanism. However, this does not seem to be the case. Despite these proposed advantages over chemotherapy, immunotherapy today still fails to deliver significant curative rates. Spontaneous and drug-resistant tumours remain virtually resistant to immunotherapy in most cancer patients (Sogn 1998).

It is clear to date that both chemotherapy and radiation mediate their cytotoxic effects through apoptosis (Figure 1). Likewise, immune lymphocytes also primarily kill by apoptosis. Thus, both share common mechanisms of killing. It is likely that the mechanism(s) for resistance of cancer cells to chemotherapy would have common or identical features with the developed resistance to apoptotic by other stimuli including immunotherapy. It follows that a strategy for an effective anti-tumour response is to utilize complimentary pro-apoptotic signals to overcome tumour resistance to immune-mediated apoptosis through the use of sensitizing agents (see review by Ng and Bonavida (2002a)). The modification of apoptosis regulatory gene products can be achieved through the use of sensitizing agents, inhibitors, antisense, siRNAs, etc. which, in combination with immunotherapy, could reverse tumour resistance.

In cancer patients, the response to treatment is dictated by many factors. The ability to stratify patients into groups that respond more positively or negatively to a given treatment would be extremely beneficial. Thus, there have been extensive efforts directed towards identifying biomarkers with such properties. Moreover, a well-characterized repertoire of biomarkers would have significant utility at every stage

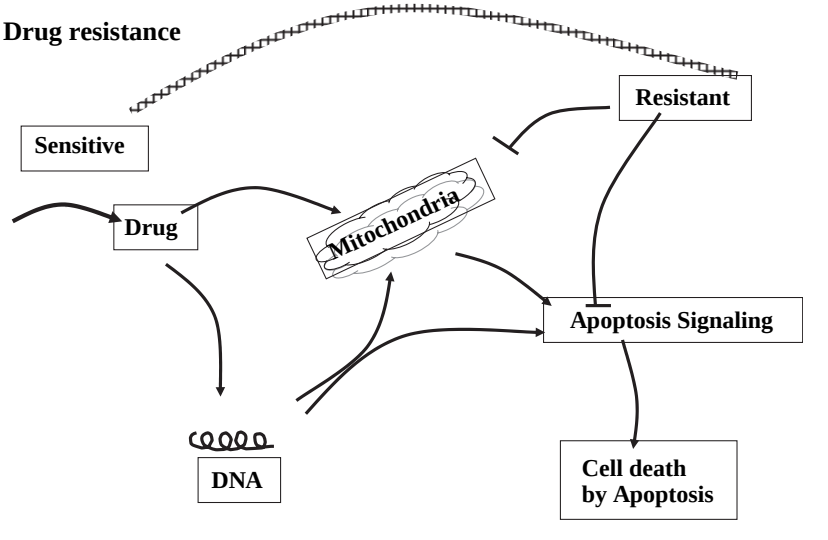

Figure 1. This diagram illustrates that chemotherapeutic drugs have many effects in the cell that culminate in cell death by apoptosis. Resistance to drugs can occur if one or more block interface with the signaling to apoptosis.

of drug development and cancer treatment. In this regard, there are many types of biomarkers, including disease biomarkers (a biomarker that relates to a clinical outcome or measure of disease), staging biomarkers (a biomarker that distinguishes between different stages of disease), efficacy biomarkers (a biomarker that reflects beneficial effects of a given treatment), etc.

Tumour cell sensitization to cytotoxic immunotherapy

Sensitization of tumour cells to cytotoxic immunotherapy involves two complementary signals (Figure 2). The first signal is 'sensitizing' and regulates pro/anti-apoptotic targets, thus facilitating the apoptotic pathway. The second apoptotic signal initiates a partial activation of the apoptotic pathway. The activation is completed by complementation with the first signal.

Identification of gene products that regulate immune resistance: New biomarkers that predict response of failure to respond to immunotherapy

Sensitizing agents that can reverse immune resistance can be used to identify a gene product(s) that regulates resistance. The expression of such gene product(s) in tumour cells may predict clinical response to immunotherapy. Examples of sensitizing agents are presented in Table I.

Studies performed in the laboratory explored several mechanisms of tumour cell resistance to immunotherapy. Figure 3 schematically demonstrates that tumour cells exhibit high basal level of constitutively activated NF-κB and that NF-κB regulates

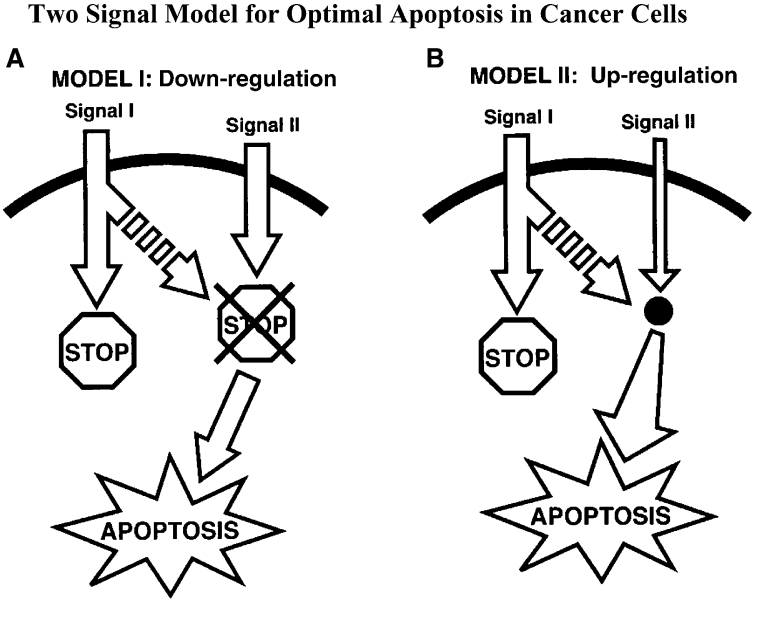

Figure 2. This model proposes that sensitizing agents (Figure 1) can either downregulate (model A) or upregulate (model B) apoptosis regulatory proteins and thus facilitates the cytotoxic agents (signal II) to mediate their apoptotic effects.

Table I. Sensitizing agents and apoptosis induced-stimuli. This table lists examples of sensitizing agents that can reverse resistance of cancer cells to either chemotherapy and/or immunotherapy-induced apoptosis. The apoptosis-inducing immune stimuli are listed and include cytotoxic lymphocytes or members of the TNF-α family.

Sensitizing agents	• Cytotoxic drugs (e.g. CDDP, VP16, ADR, ActD) • Biologic factors (e.g. INF-γ) • Antibodies (e.g. Rituximab (anti-CD20)) • Nitric oxide donors
Apoptosis-inducing cells/factors	• Cytotoxic lymphocytes (CTL, NK) • Recombinant ligands, FasL, TNF-α, TRAIL

many gene products including several anti-apoptotic and inflammatory cytokines. Agents that can inhibit NF-κB can regulate sensitivity to immune-mediated apoptosis (e.g. TNF-α, Fas L, TRAIL) via inhibition of the transcription repressor Ying-Yang-1 (YY1) (Garban & Bonavida 2001). YY1 can be inhibited both by siRNA and NO and its inhibition upregulates the expression of the immune receptors and sensitizes cells to immune-mediate apoptosis.

Studies have identified gene products whose expression regulate tumour cell sensitization to cytotoxic immunotherapy (Table II). These include transcription factors such as NF-κB, YY1, AP-1, anti-apoptotic gene products such as Bcl-2, Bcl-$_{xL}$, XIAP and pro-apoptotic products such as Smac/DIABLO, DR5, RKIP (Huerta-Yepez et al. 2004, Jazirehi et al. 2004, Vega et al. 2004).

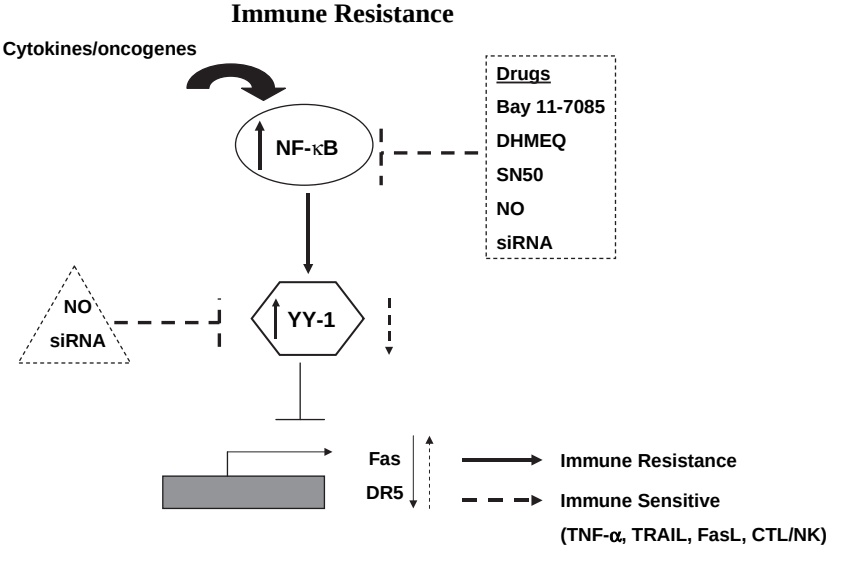

Figure 3. This figure schematically illustrates that tumour cell resistance to immune-mediated apoptosis is the result of several potential mechanisms. The studies have focused on the role of constitutively activated NF-κB in tumour cells and that serves as an anti-apoptotic factor and its inhibition sensitizes the cells to immune-mediated apoptosis. The mechanism by which NF-κB sensitizes tumour cells to apoptosis was examined and was found that YY1 under the transcription regulation of NF-κB plays an important role in the regulation of resistance through the negative regulation of the transcription of immune receptors (TNF-α, FasL and TRAIL). Like inhibition of NF-κB, inhibition of YY1 also sensitizes the cells to immune-mediated apoptosis via upregulation of immune receptors.

Table II. Identification of potential markers for immunotherapy. This table lists a few examples of the underlying mechanisms by which the sensitizing agents function to reverse resistance. The sensitizing agents modify the expression/activity of gene products that regulate resistance. These gene products are potential biomarkers for analysis.

Examples	Biomarkers
• Sensitization to CTL mediated killing	YY1; Bcl-$_{xL}$; Smac/DIABLO, NF-κB,
• Sensitization to Fas/TNF-α/TRAIL-induced apoptosis	AP-1, p38 MAPK
• Sensitization to antibody-mediated apoptosis	Bcl-2; Bcl-$_{xL}$; RKIP; survival pathways

Examples of functional gene products in tumour cell resistance to immunotherapy that might also be useful biomarkers

NF-κB

The NF-κB family of dimeric transcription factors has been shown to modulate cell survival during stress and immune responses (Baeuerle & Baltimore 1996). NF-κB protects cells from apoptosis by promoting expression of survival factors (Wang et al. 1996, 1998). NF-κB also protects cells from immune-mediated apoptosis (Ravi et al. 2001, Huerta-Yepez et al. 2004). Thus, high expression of NF-κB in the nucleus of the tumour may suggest a hyperactivation of anti-apoptotic regulatory gene products and resistance to immune-mediated-apoptosis.

YY1

The transcription repressor (YY1) has been shown to negatively regulate Fas expression in cancer cells and contributes to tumour cell resistance in response to Fas-mediated apoptosis (Garban & Bonavida 2001). Further, recent findings suggest that YY1 also regulates tumour cell resistance to TRAIL-induced apoptosis in prostate cancer cells (Huerta-Yepez et al. unpublished). Finally, YY1 has been shown to be over-expressed in human prostate cancer tissues compared to non-malignant tissue, as measured by tissue micro-array analysis. YY1 expression also appears to have prognostic significance (Seligson et al. 2005). Therefore, the expression of YY1 in tumour tissues may predict response to immunotherapy.

Smac/DIABLO

The TNF ligand super-family plays an important role in the host immune defense against cancer as an anti-tumour death inducing agent (Nagata 1997). This super-family induces cell death by apoptosis in sensitive target cells by the death receptor pathway. The apoptotic signalling pathway is subjected to several levels of inhibition by regulation (Ashkenazi & Dixit 1999). Tumour cells over-express inhibitory and anti-apoptotic proteins (IAPs) (Deveraux et al. 1998) and a mitochondrial molecule, Smac/DIABLO, has been documented to be a neutralizing inhibitor of the anti-apoptotic IAP family of proteins (Du et al. 2000). Thus, tumour cells that express low levels of Smac/DIABLO may be more resistant to immune-mediated apoptosis than cells over-expressing Smac/DIABLO. Indeed, *in vitro*, it is demonstrated that prostate cancer cells resistant to TRAIL can be sensitized by over-expression of Smac/DIABLO (Ng & Bonavida 2002b). In cancer patients, it has recently been

demonstrated that low expression of Smac/DIABLO in renal cancer tumours predicted worse prognosis and survival (Mizutani et al. 2004).

Soluble Fas and soluble Fas ligand

The receptor Fas expressed on the surface of tumour cells can be triggered by the Fas ligand (FasL) expressed on cytotoxic lymphocytes and results in apoptosis of the Fas-sensitive cancer cells (Kagi et al. 1994). While both Fas and FasL are predominately integral membrane proteins, both can also be expressed in soluble, secreted forms. Production of these soluble variants is potentially one survival strategy by tumour cells. At the same time, one may be able to take advantage of this by detecting these products as tumour markers. Soluble Fas (sFas) is generated by alternative mRNA splicing events. As Fas can bind to FasL, Fas secretion may be one of the mechanisms responsible for tumour cell resistance to Fas-mediated apoptosis. Soluble FasL (sFasL) is produced by a different mechanism. Cleavage of membrane-bound FasL by a metalloprotease-like enzyme results in the generation of soluble FasL (Tanaka et al. 1996). Similar to membrane bound FasL, sFasL can also transduce an apoptotic signal in Fas-expressing sensitive cells (Tanaka et al. 1995). However, FasL has been reported to be a weaker inducer of apoptosis compared to membrane bound FasL (Tanaka et al. 1998). Thus, in contrast to membrane FasL, sFasL can protect cells from Fas-mediated apoptosis (Suda et al. 1997). Secreted levels of sFas (Mizutani et al. 1998) and FasL (Mizutani et al. 2001) have been reported to be of prognostic significance in patients with bladder cancer. Further, a combination of serum levels of sFas and sFasL in patients with bladder cancer predicted recurrence after trans-urethral resection (Mizutani et al. 2002). These findings strongly suggest that sFas and sFasL levels can be used as prognostic markers for tumour recurrence

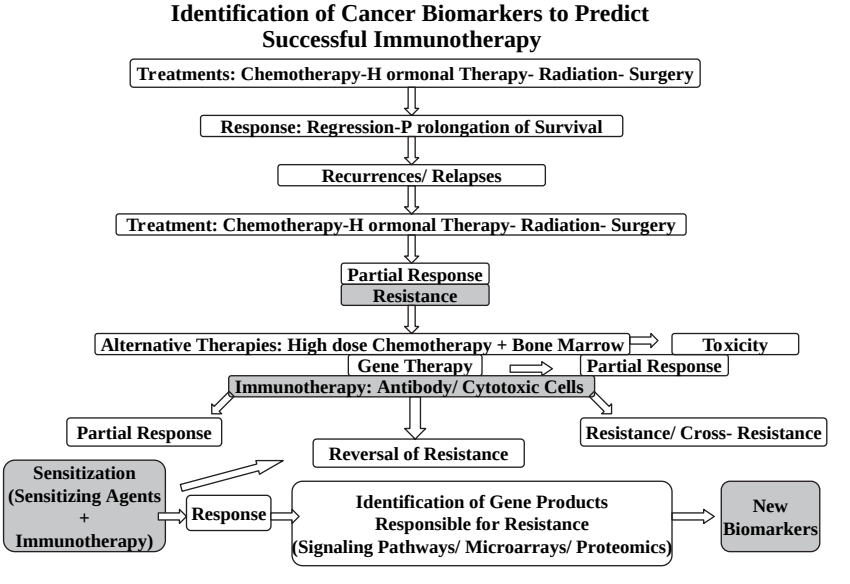

Figure 4. This scheme illustrates the methods used to identify biomarkers of clinical significance for response of resistant cancer cells to immunotherapy.

Concluding remarks

The development of biomarkers for successful immunotherapy is extremely beneficial to stratify the patient population in order to increase their response rate. Oncology is among the first areas to reap benefits of biomarker research, both in terms of diagnosis and treatment. Cancer is often a terminal disease where, if appropriate treatment is not decided quickly, the window of opportunity to treat the disease effectively may be lost. Also, there is increasing number of new oncology medicines and therapeutic choices that can be facilitated by diagnostics to better clarify the type of cancer and choose appropriate treatment. Unlike biomarkers for drug resistance, there have not been many biomarkers for immune resistance and these need to be characterized and validated in the clinical setting. The immune biomarkers examples provided in this report are the first to be completed and to be validated in the clinical setting. A general scheme for the characterization of an immune biomarker is illustrated in Figure 4. While the use of biomarkers in human studies is new, biomarkers are used to help make decisions to select the most promising candidates and/or identify the right patients for particular treatments.

Acknowledgements

This work was supported in part by DOD/US ARMY DAMD 17-02-1-0023 (BB) and the Early Detection Research Network (LG) NCI CA-86366. We acknowledge the assistance of Dr D. Seligson and S. Horvath for analyses of prostate cancer tissue micro-arrays for YY1 expression and we thank Ms Christine Yue for the preparation of this manuscript.

References

Ashkenazi A, Dixit VM. 1999. Apoptosis control by death and decoy receptors. Current Opinions in Cell Biology 11:255–260.

Baeuerle PA, Baltimore D. 1996. NF-kappa B: ten years after. Cell 87:13–20.

Deveraux QL, Roy N, Stennicke HR, Van Arsdale T, Zhou Q, Srinivasula SM, Alnemri ES, Salvesen GS, Reed JC. 1998. IAPs block apoptotic events induced by caspase-8 and cytochrome c by direct inhibition of distinct caspases. EMBO Journal 17:2215–2223.

Du C, Fang M, Li Y, Li L, Wang X. 2000. Smac, a mitochondrial protein that promotes cytochrome c-dependent caspase activation by eliminating IAP inhibition. Cell 102:33–42.

Garban HJ, Bonavida B. 2001. Nitric oxide inhibits the transcription repressor Yin-Yang 1 binding activity at the silencer region of the Fas promoter: a pivotal role for nitric oxide in the up-regulation of Fas gene expression in human tumor cells. Journal of Immunology 167:75–81.

Huerta-Yepez S, Vega M, Jazirehi A, Garban H, Hongo F, Cheng G, Bonavida B. 2004. Nitric oxide sensitizes prostate carcinoma cell lines to TRAIL-mediated apoptosis via inactivation of NF-kappa B and inhibition of Bcl-xl expression. Oncogene 23:4993–5003.

Jazirehi AR, Vega MI, Chatterjee D, Goodglick L, Bonavida B. 2004. Inhibition of the Raf-MEK1/2-ERK1/2 signaling pathway, Bcl-xL down-regulation, and chemosensitization of non-Hodgkin's lymphoma B cells by Rituximab. Cancer Research 64:7117–7126.

Kagi D, Vignaux F, Ledermann B, Burki K, Depraetere V, Nagata S, Hengartner H, Golstein P. 1994. Fas and perforin pathways as major mechanisms of T cell-mediated cytotoxicity. Science 265:528–350.

Mizutani Y, Hongo F, Sato N, Ogawa O, Yoshida O, Miki T. 2001. Significance of serum soluble Fas ligand in patients with bladder carcinoma. Cancer 92:287–293.

Mizutani Y, Nakaishi H, Yamamoto K, Nan Li Y, Matsubara H, Mikami K, Okihara K, Kawauchi A, Bonavida B, Miki T. 2005. Downregulation of Smac/DIABLO expression in renal cell carcinoma and its prognostic significance. Journal of Clinical Oncology 28:448–454.

Mizutani Y, Yoshida O, Bonavida B. 1998. Prognostic significance of soluble Fas in the serum of patients with bladder cancer. Journal of Urology 160:571–576.

Mizutani Y, Yoshida O, Ukimura O, Kawauchi A, Bonavida B, Miki T. 2002. Prognostic significance of a combination of soluble Fas and soluble Fas ligand in the serum of patients with Ta bladder cancer. Cancer Biotherapy & Radiopharmaceutics 17:563–567.

Nagata S. 1997. Apoptosis by death factor. Cell 88:355–365.

Ng CP, Bonavida B. 2002a. A new challenge for successful immunotherapy by tumors that are resistant to apoptosis: two complementary signals to overcome cross-resistance. Advanced Cancer Research 85:145–174.

Ng CP, Bonavida B. 2002b. X-linked inhibitor of apoptosis (XIAP) blocks Apo2 ligand/tumor necrosis factor-related apoptosis-inducing ligand-mediated apoptosis of prostate cancer cells in the presence of mitochondrial activation: sensitization by overexpression of second mitochondria-derived activator of caspase/direct IAP-binding protein with low pl (Smac/DIABLO). Molecular Cancer Therapy 1:1051–1058.

Patel NH, Rothenberg ML. 1994. Multidrug resistance in cancer chemotherapy. Investigative New Drugs 12:1–13.

Pommier Y, Sordet O, Antony S, Hayward RL, Kohn KW. 2004. Apoptosis defects and chemotherapy resistance: molecular interaction maps and networks. Oncogene 23:2934–2949.

Ravi R, Bedi GC, Engstrom LW, Zeng Q, Mookerjee B, Gelinas C, Fuchs EJ, Bedi A. 2001. Regulation of death receptor expression and TRAIL/Apo2L-induced apoptosis by NF-kappaB. National Cell Biology 3:409–416.

Seligson D, Horvath S, Huerta-Yepez S, Hanna S, Garban H, Roberts A, Shi T, Liu X, Chia D, Goodglick L, Bonavida B. 2005. Expression of transcription factor Ying-Yang 1 in prostate cancer. International Journal of Oncology 27:131–141.

Sogn JA. 1998. Tumor immunology: the glass is half full. Immunity 9:757–763.

Suda T, Hashimoto H, Tanaka M, Ochi T, Nagata S. 1997. Membrane Fas ligand kills human peripheral blood T lymphocytes, and soluble Fas ligand blocks the killing. Journal of Experimental Medicine 186:2045–2050.

Tan B, Piwnica-Worms D, Ratner L. 2000. Multidrug resistance transporters and modulation. Current Opinions in Oncology 12:450–458.

Tanaka M, Itai T, Adachi M, Nagata S. 1998. Downregulation of Fas ligand by shedding. Natural Medicine 4:31–36.

Tanaka M, Suda T, Haze K, Nakamura N, Sato K, Kimura F, Motoyoshi K, Mizuki M, Tagawa S, Ohga S, Hatake K, Drummond AH, Nagata S. 1996. Fas ligand in human serum. Natural Medicine 2:317–322.

Tanaka M, Suda T, Takahashi T, Nagata S. 1995. Expression of the functional soluble form of human fas ligand in activated lymphocytes. EMBO Journal 14:1129–1135.

Vega MI, Huerta-Yepaz S, Garban H, Jazirehi A, Emmanouilides C, Bonavida B. 2004. Rituximab inhibits p38 MAPK activity in 2F7 B NHL and decreases IL-10 transcription: pivotal role of p38 MAPK in drug resistance. Oncogene 23:3530–3540.

Wang CY, Mayo MW, Baldwin AS Jr. 1996. TNF- and cancer therapy-induced apoptosis: potentiation by inhibition of NF-kappaB. Science 274:784–787.

Wang CY, Mayo MW, Korneluk RG, Goeddel DV, Baldwin AS Jr. 1998. NF-kappaB antiapoptosis: induction of TRAF1 and TRAF2 and c-IAP1 and c-IAP2 to suppress caspase-8 activation. Science 281:1680–1683.