My research interests lie in two quite different fields of science: 1) tumor associated antigens (TAA) and the antibodies directed against them, and 2) marine mammal vision and the associated visual pigments.

1) Tumor Associated Antigens

The use of tumor extracts as crude vaccines to stimulate an immune response to cancer was pioneered as a treatment option over 30 years ago (Gilboa, 2004). Recent attempts along these lines have included augmenting whole cell vaccines with a variety of tumor specific peptides expressed by the cancer along with genetic engineering to express known immuno-stimulatory molecules. The rationale behind this approach is to try to enhance the in vivo delivery of tumor antigens to cells of the immune system in the face of an inherently weak host response to cancer cells. Likewise, there are currently several commercially available monoclonal antibodies (mAbs) approved for cancer therapy (Herceptin, Erbitux, Rituxan, Campath and Avastin), each of which has proved remarkably effective against a variety of malignancies (e.g. breast, large bowel, lung, lymphoma, leukemia). These initial victories in the battle against cancer, however, are enormously overshadowed by the lack of knowledge in regards to the countless uncharacterized cancer candidate genes. Recent work by Sjöblom et al. (2006) has shown that, in breast and colorectal cancers alone, there exists almost 200 genes mutated at significant frequency which contribute to the neoplastic process. Furthermore, each individual tumor has on average a dozen of these mutations. Results such as these demonstrate the continuing need to understand the genetics of cancer. With these genetic advances will come discoveries uncovering how TAAs are regulated and how they can be targeted for tumor cell destruction.

My approach to these problems is multi-disciplinary combining genetics, immunology, molecular biology, and tissue culture. The following projects are designed to further investigate known TAAs as well as to identify new ones. These projects may also lead to the development of new mAbs that may be useful either as diagnostic agents or as immunotherapeutics.

Project 1 will investigate the therapeutic potential of the mAb 33.28. This mAb was initially cloned based on its ability to bind well to some colon and pancreatic tumor cell lines. To elucidate its value as a potential therapeutic agent, various assays including antibody dependent cellular cytotoxicity (ADCC), complement dependent cytotoxicity (CDC), clonogenic assays, as well as cell based binding kinetics will be performed. Antigen identification can be done using various techniques including immunoprecipitation, MALDI-MS, Edman sequencing and sequence comparison to protein databases. If this mAb proves to be novel and results in some form of tumor cell degeneration, genetic engineering can be used to humanize it for use in patients.

Project 2 will investigate the effect chemotherapeutic drugs have on TAA induction. Cancer cells differ from normal cells in their response to chemotherapy. Previous experiments with colorectal cancer have exploited this dissimilarity and
identified tumor-specific, cell surface proteins whose expression is induced by chemotherapeutic agents (Rubinfeld et al. 2006). I would like to take a similar approach in a study of pancreatic cancer. This involves culturing various pancreatic tumor cell lines in combination with chemotherapeutic agents (e.g. gemcitabine) and then comparing oligonucleotide microarray analysis of RNA purified from treated and untreated tumor cell lines. Upregulated transcripts can be targeted for amplification and expression for the development of immunotherapeutic agents as well as lay the foundation for future studies.

Project 3 will examine the genetics of cancer. Many colon and pancreatic cancers result in the overexpression of the human glycoprotein A33. A33 is a surface protein believed to be involved with cell adhesion. A33 has been shown to be an excellent TAA for immunotherapy, but very little is known of the regulation and upstream and downstream associations of this protein. In order to elucidate the role A33 plays in cancer the following investigations can be done. 1) Total genome microarray analysis of tumor cell lines heterogeneous for A33 expression. In these investigations the two genomes are compared for their differences in copy number on a microarray. Identification of amplified and deleted genes may lead to pathways by which A33 is overexpressed as well as to the effect that overexpression has on other genes; 2) Complementary DNA microarray analysis of A33 expressing tumor cells bound and unbound with monoclonal antibody. It has been shown that the binding of antibody to the A33 antigen results in cell death in the absence of both effector cells (ADCC) and serum complement (CDC). This indicates that the binding of antibody to antigen may result in an apoptotic event. cDNA microarray analysis will be used in determining the pattern of gene expression and may identify those genes involved in cell death.

Project 4 will be the development of a number of anti-cancer antibodies using previously characterized TAA preparations from malignant tissues (Arlen et al., 1998; Hollinshead et al., 1985). These antigens are, for the most part, immunogenic glycoproteins. TAAs were previously screened by a delayed hypersensitivity response in patients after receiving a subcutaneous injection of a partially purified carcinoma membrane preparation (Hollinshead et al., 1985; Arlen et al., 1998). These positive fractions will be used to develop hybridomas that are then screened and cloned against a large panel of tumor cell lines. Novel TAAs may be identified by various techniques including immunoprecipitation, MALDI-MS, Edman sequencing and sequence comparison.

2) Marine Mammal Vision

The vertebrate eye has been a center-piece of evolutionary speculation. It's interacting series of morphological features and protein components have been remarked as one of the most astounding examples of evolution by natural selection. Experimental studies of genes that code for the light-gathering pigments in vertebrates have shown how the properties of these genes are tuned to the environments in which species live, suggesting that adaptation extends even to the molecular level in this structure. Some groups of mammals have shown marked ecological shifts in habitat that require alterations in how the eye functions. No better example of this can be found amongst the
mammals than the evolutionary return to the sea of the cetaceans, whales and dolphins. The cetacean eye has not shed its terrestrial modification but rather has accumulated traits quite divergent from those of terrestrial mammals.

Previous studies (Fasick & Robinson, 1998; Fasick & Robinson, 2000; Fasick et al. 1998) were designed to assess the bottlenose dolphin's (*Tursiops truncatus*) capacity for color vision and determine the absorption maxima of the dolphin visual pigments. To this end, the dolphin opsin genes were cloned and expressed. The resulting proteins were reconstituted with the chromophore 11-cis-retinal resulting in functional pigments with absorption maxima of 488 and 524 nm for the rod and cone pigments respectively. These values are considerably blue shifted compared to those of many terrestrial mammals. Although the dolphin possesses a gene homologous to other mammalian short-wavelength sensitive (SWS) opsins, it is not expressed *in vivo* and has accumulated a number of deletions, including a frame-shift mutation at nucleotide position 31. The dolphin therefore lacks the common dichromatic form of color vision typical of most terrestrial mammals.

A comparison of the sequence of the dolphin rod photopigment gene with that of the bovine rod suggests that, of the 28 nonidentical amino acids, three amino acid substitutions at positions 83, 292, and 299 in the dolphin rod pigment are responsible for the 10 nm blue shift in absorption maxima. A similar comparison of the dolphin long-wavelength sensitive (LWS) cone photopigment gene with those of the human LWS cones suggests that a single substitution at position 292 (using the convention of rhodopsin numbering) in the dolphin LWS cone pigment results in a blue shift in absorption maxima. A mutagenesis study reveals that the combination of the three dolphin specific substitutions in the bovine rod pigment (83D to 83N, 292A to 292S, and 299A to 299S) causes a blue shift from the wild-type absorption maximum of 499 nm to 389 nm. The single substitution in the dolphin LWS cone pigment (292S to 292A) causes a red shift from the wild-type absorption maximum of 524 nm to 552 nm. The interactions of the three amino acids identified in the rod pigment with the chromophore may be a general mechanism for blue shifting in rod visual pigments. Furthermore, the single substitution in the dolphin LWS opsin gene is a novel mechanism of wavelength modulation in mammalian LWS pigments.

The mutagenesis information above was used in a study to investigate the molecular basis for changes in the spectral sensitivity of rod visual pigments from seven distantly related marine mammals. The results show a relationship between blue-shifted rhodopsins (absorption maxima < or = 490 nm), deep-diving foraging behavior, and the substitutions 83Asn and 292Ser. Species that forage primarily near the surface in coastal habitats have a rhodopsin with an absorption maximum similar to that of terrestrial mammals (500 nm) and possess the substitutions 83Asp and 292Ala, identical to rhodopsins from terrestrial mammals.

Future studies will examine the role of melanopsin in the marine mammal eye. Melanopsin is a light sensitive pigment associated with the retinal ganglion cell layer of the vertebrate eye. Unlike the cone and rod visual pigments, melanopsin is not associated
with image formation, but rather has been associated with physiological responses such as circadian photoentrainment and the pupilary light response.

References


