Meeting Report

Differentiation or Death: The View from Versailles

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The Seventh International Conference on Differentiation Therapy of Cancer
Versailles, France 5–8, October, 1997

Abbreviations: ATRA, all trans retinoic acid; APL, acute promyelocytic leukemia; AML, acute myelogenous leukemia; HMBA, Hexamethylene bisacetamide; Interferon A ($\text{INF}\alpha$); RAR, retinoic acid receptors; RXR, retinoic acid X receptors

More effective, less toxicity therapy is a wish frequently voiced by clinical oncologists faced with patients who have failed conventional therapies. With an eye towards making this wish a reality, over the last 12 years the Conference on Differentiation Therapy of Cancer has teamed clinical scientists with basic research scientists in order to stimulate scientific exchange and collaborations with the ultimate goal to develop novel therapeutic approaches in oncology based on modulating the signal transduction paths that control cell proliferation and differentiation. With the appreciation that induction of apoptosis is a developmental process gone awry in cancer cells one of the major areas of interest that emerged from the 7th International Conference on Differentiation Therapy was the development of therapeutic modalities based on induction of apoptotic signal transduction pathways.

APL

One of the major successes of differentiation therapy was the finding that acute promyelocytic leukemia (APL, AML-M3 and M3-variant) marked by a chromosomal translocation $t(15;17)$ resulting in chimeric proteins involving retinoic acid receptors are sensitive to differentiation induction by all-trans retinoic acid (ATRA). ATRA has made major improvements in survival with minimal side effects in patients with this disease, however, relapse and resistance to ATRA still occur. Clinical studies using ATRA in combination with chemotherapy, novel retinoids and arsenate indicated promising new treatment modalities. R. Ohno (Hamamatsu) presented studies on newly diagnosed patients in which ATRA alone or in combination with daunorubicin and behenoyl ara-C were utilized. In 196 evaluable patients, they found 88% achieved a complete remission (CR) with a predicted 4 year disease-free survival (DFS) at 62% and 54% for event-free survival (EFS). Low WBC ($<10\,000/\mu\text{l}$) was a significant prognostic factor for a longer DFS while age (<30 years) was a significant factor for longer EFS. P. Fenaux presented the European APL Group APL91 trial in which patients were randomized to ATRA followed by chemotherapy or chemotherapy alone. While the CR rate was comparable between the two arms,

there was clearly a difference in the relapse rates with 31% of those receiving ATRA relapsing at 4 years compared to 72% relapse with chemotherapy alone. ATRA/chemotherapy was superior to chemotherapy alone in both EFS (62% vs 15%) and overall survival (76% vs 49%). A preliminary report on the APL93 trial in which ATRA concomitant with chemotherapy or ATRA followed by chemotherapy was evaluated. From 400 patients they found that induction with ATRA and chemotherapy yielded greater than 90% CR and that the schedule of ATRA concomitant with chemotherapy was more effective than sequential chemotherapy and the use of maintenance therapy was also beneficial in terms of relapse. As S. Waxman (New York) pointed out in a plenary session, an important issue in the application of differentiation based therapies is the development of preclinical models to test issues related to drug scheduling. In an APL preclinical model, it was shown that combination cytotoxic-differentiation therapy was schedule dependent as a priming dose of RA rendered cells capable of differentiation when induced by agents such as Vitamin D, Hexamethylbisacetamide (HMBA) or butyrate which alone had little effect. He proposed that strategies integrating these types of approaches should be used in combination with efforts such as the NCI Drug Screen.

Of the more intriguing treatments discussed for relapsed APL was the use of arsenical compounds. Z. Chen (Shanghai) presented data that 24/25 of relapsed APL patients receiving AS_2O_3 at 10 mg/ml/day (some with other chemotherapeutic agents) for 1–2 months achieved a CR with some of the 16 patients in CR up to 24 months. Clearly a setting in which arsenate will be useful is in patients who have relapsed after ATRA therapy or who have poor prognostic indicators. In preclinical studies W. Miller (Montreal) found that arsenate decreased PML-RAR α protein and induced apoptosis in an ATRA resistant subline of the APL cell line NB4. R. Warrell (New York) found that cell lines from patients with Chronic Lymphocytic Leukemia (CLL) as well as Chronic Myelogenous Leukemia (CML) were sensitive to the arsenate and Phase I studies will proceed using AS_2O_3 . Whether AS_2O_3 will have activity in other tumors awaits preclinical testing.

APL is characterized by chromosomal translocations that juxtapose the RAR α gene to a number of other genes such as PML, PLZF, NPM and NuMA. J. Licht and P-P. Pandolfi (New York) presented elegant transgenic studies clearly implicating expression of the PML-RAR α or PLZF-RAR α

chimeric proteins in the leukemogenic process. Using the cathespin promoter which is highly expressed in promyelocytes, APL chimeric proteins were expressed in transgenic mice. All of the transgenics expressing the PML-RAR α transgene developed a myeloid hyperproliferative disease with 10% showing leukemia while all of the transgenics expressing the PLZF-RAR α fusion gene developed leukemia. A better understanding of the mechanisms of transformation of the PLZF chimeric protein which is important especially since patients carrying this translocation have a worse prognosis and are not sensitive to retinoids. F. Guidez (London) found that the activity of the different retinoic acid receptor chimeric proteins in APL may be influenced by their differential interaction with co-regulators such as N-CoR or SMRT. The mechanism by which these chimeric proteins transform these cells is still unclear. Of interest in this regard is the curious story of the nuclear bodies (normal nuclear structures with unknown functions) in APL. ATRA treatment of APL cell lines with the t(15:17) causes degradation of the PML-RAR α fusion gene and restoration of nuclear bodies. H. de Thé (Paris) found that arsenate treatment was also associated with the targeting of the PML-RAR α to the nuclear bodies and the subsequent degradation of the fusion product and restoration of the nuclear body structure. Unfortunately the lack of understanding of the nature and role of these nuclear structures in cell biology has limited the understanding how these clinically active agents may be targeting cells for induction of differentiation and programmed cell death.

The efficacy of ATRA in PML has prompted a number of clinical trials in other hematopoietic malignancies as well as in solid tumors. ATRA in combination with Interferon A (INFa) had minimal activity in multiple myeloma. Reports on small groups of patients (20–25) by T. Dorval (Paris) and B. Dreno (Nantes) indicated that ATRA and INFa had modest activity in malignant melanoma. S. Philippe (Paris) found ATRA alone was well tolerated, did not alter viral loads and caused a PR or stabilization of lesions in most low risk AIDS-KS patients on the study.

Aromatic fatty acids

Evidence was also presented that another member of the steroid family of nuclear receptors may play a major role in the activity of compounds used in Differentiation Therapy. D. Samid (Charlottesville) indicated that a potential mechanism of action for aromatic fatty acids may be via activation of the peroxisome-proliferator activated receptors (PPAR). PPAR are thought to play a role in the control of lipid metabolism. The differentiation and induction of apoptosis stimulated by the class of compounds called aromatic fatty acids (which includes phenylbutyrate and its metabolite phenylacetate) has been proposed to be mediated by glutamine starvation, inhibition of protein isoprenylation and perhaps farnesylation, alteration of lipid metabolism and effects on chromatin structure. M. Carducci (Baltimore) also found that phenylbutyrate altered histone acetylation and decreased telomerase activity in prostate cancer cell lines. In Phase I studies of other adult solid tumors, evidence for stabilization of disease or an

improvement of overall well being was reported. Dosing with either continuous infusion for 120 h every 21 days or oral thrice daily chronic dosage. Dose limiting toxicity (400 mg/kg/day) on the infusional study was confusion or somnolence which was reversible while the oral dosing continues to be escalated. Preclinical data in leukemic cell lines as well as primary cultures of AML and MDS indicated significant cytostatic and differentiation activity leading to a current Phase I clinical trial in AML and MDS discussed by S. Gore (Baltimore). Similarly preclinical activity in brain tumor cell lines has led to a Phase I protocol in adults as well as children at the NCI.

Hybrid polar compounds (HPC)

Hexamethylene bisacetamide (HMBA) is the prototype of the HPC class of compounds that are potent inducers of differentiation and apoptosis in a number of tumor cell lines. P. Marks (New York) presented studies showing HPC induce a prolongation of G1 associated with a G1 arrest that occurs stochastically after 4–6 replicative cycles in a murine erythroleukemia cell line. HPC effects on the cell cycle machinery may be cell type specific. V. Richon (New York) showed data that HPCs induce differentiation in bladder carcinoma cell lines if E2F/pocket protein complexes are formed but induce apoptosis when E2F/pocket proteins fail to complex either due to mutations in Rb or expression of human papilloma virus 16-E7 which binds Rb. J. Michaeli (New York) showed in melanoma cells that the HMBA induced reduction of the anti-apoptotic gene bcl-2 was associated with induction of apoptosis. The identification of a group of HPC's that are more than 2000-fold active than HMBA fuels continued interest in the development of these compounds for Phase I/II testing.

Novel retinoids

The complexities of the retinoid nuclear receptor signaling mechanisms were presented by D. Metzger (Strausborg) who reviewed the data on the RAR and RXT KO mice and the double RAR $\times$ RXR KO mice. Parallel studies using receptor selective agonists and antagonists and clones of the F9 murine embryonal carcinoma cell line null for expression of the isoforms of RAR alone or in combination with RXR have been used to attempt to dissect the RAR;RXR signaling paths important in controlling growth and differentiation. F9 cells lacking RXR expression showed decreases in proliferation but one of the more striking results presented was the observation that in the RAR γ ; RXR null F9 cells; RA not only fails to arrest growth but may actually stimulate proliferation. To pinpoint the role of RAR:RXR in particular cell types, A. Kakizuka (Kyoto) developed transgenic animals containing a dominant negative form of RAR driven by tissue specific promoters. Specific expression of the dominant negative RAR in the epidermis resulted in skin lacking keratinization while expression in chondrocytes caused a partial loss of cranial bones in the heterozygotes with more extensive losses in homozygous mice. Although progress is being made in delineating the myriad of effects of activation of retinoic acid receptors, it is clear that the functional redundancy of the

system makes it a puzzle that will only painstakingly be pieced together.

Retinoid receptor selective agonists and antagonists have been used to identify compounds that may have greater therapeutic utility or more selective activity in different cell lineages. M. Dawson (Menlo Park) found agonists that increased RAR β expression in addition to activation of RXR were effective in inhibiting the growth of small cell lung cancer cell lines. X. Zhang (La Jolla) found induction of RAR β either by RAR selective retinoids or agonists that activated RXR:RAR α were effective in controlling the growth of breast cancer cell lines *in vitro*. C. Thiele (Bethesda) reported that agonists leading to activation of RXR:RAR and particularly RXR:RAR β heterodimers were effective in controlling growth in neuroblastoma cells and consistent with this observation was the study of A. Carpentier (Paris) that showed an increase in RXR:RAR β heterodimer formation on model RAREs in RA treated NB cells. C. Redfern (Newcastle) presented data that 9CRA may be more effective in inducing apoptosis in certain morphologic variants of neuroblastoma. Investigators were urged to exercise caution in the interpretation of results utilizing retinoid agonists and antagonists since frequently reported biologic effects occurred at concentrations of these retinoids in which there was no receptor selectivity. Further complicating the interpretation of the activity of these agents was that some compounds may have different receptor selectivities depending on the promoter context used in their characterization.

The real interest in the retinoid field lay in those compounds that induced apoptosis such as 6-[3-(1-adamantyl)-4-hydropyphenyl]-2-naphthalene carboxylic acid (CD437) and 4-(N-hydroxyphenyl) retinamide (4HPR). CD437 was reported to induce apoptosis in both estrogen receptor positive and negative breast cancer cells (M. Dawson, Menlo Park); lung and lymphoma cancer cell lines (A. Jetten, Research Triangle Park) and the NB4 APL cell line (E. Garattini, Milan and R. Lotan, Houston). 4HPR induction of apoptosis in some cell types may be blocked by RAR-specific antagonists indicating involvement of nuclear receptors while in other cell types the induction of apoptosis by 4HPR may depend on the generation of reactive oxygen species. CD437 induced apoptosis in lymphoma cells was proposed to be mediated by both caspase dependent and independent mechanisms and in carcinoma cells the action of CD437 was clearly not suppressed by retinoic acid receptor antagonists. How these compounds activate the apoptotic signal transduction paths is still unknown.

Novel approaches

As consequence of the activation of oncogenes or the loss of tumor suppressor genes or mutator genes the expression of a

large number of normal genes is aberrantly regulated in cancer cells. In a Workshop on 'Genes that promote and interfere with terminal cell divisions and programmed cell death', the chairs P. Fisher (New York), M. Lanotte (Paris) and D. Levens (Bethesda) and participants outlined how current technologies for gene discovery such as microchip technologies, antisense strategies, differential display could identify important genes regulating physiologically important processes. Studies on altered transcription factors in T-cell acute leukemia (T. Rabbitts, Cambridge), abnormal cyclin A expression (C. Brechot, Paris) a myeloid transcription factor (H.P. Koeffler, Los Angeles) indicated that the study of normal and abnormal gene regulation may lead to new strategies for intervention. By decreasing expression of MCL1, a BCL2 family member, R. Craig (Hanover) induced an apoptotic rather than differentiation program in myeloid cells. Many biologic response modifiers are thought to act by complementing abnormal genetic programs in tumor cells to induce growth control and differentiation. A. Pardee (Boston) presented an interesting view of this process as he discussed how differential display data from R. Sager's lab could be used as a model in which to search for drugs or develop other interventions that restored regulated gene expression. Newer strategies may be aimed at gene reconstitution by specific targeting of abnormal genes by antisense, ribozymes or dominant negative proteins. To this point, utilizing specific anti-sense knockout experiments, A. Kimchi (Rehovot) discussed the analyses of genes encoding Death Associated Proteins (DAP) that block interferon gamma induced apoptosis. One such gene is DAP3, a gene which encodes a novel calcium/calmodulin kinase gene that interacts with actin filaments and is expressed in many tumor types. Interestingly, in metastatic and non-metastatic tumor variants, the DAP3 kinase is expressed in non-metastatic variants, yet not in highly metastatic tumors. Reintroduction of the DAP3 kinase into a metastatic variant suppressed the metastatic capability of the cells. A gene that functionally suppresses apoptosis and metastasis raises the possibility of direct links between the signaling paths mediating these processes.

As exemplified by the use of ATRA in APL, much progress has been made in the development of novel agents with greater efficacy and less toxicity. However a broader applicability of this mode of therapy requires a better understanding of the mechanism by which differentiation or apoptosis inducing agents act and how other compounds might be used to exploit these processes in tumors from different cell lineages. We look forward to the Eighth International Conference on Differentiation Therapy to be held in Montreal in 1999 in anticipation that the analysis of strategies employing Differentiation Therapy will have a greater impact on the treatment of cancer.