departments or faculties in universities or medical centers. Another option would provide government contracts to commercial laboratories to produce such products. Finally, governments might encourage corporations to more actively pursue these strategies by offering patent extensions or other incentives.

Recognizing the importance of promoting investigations of immunotherapy combinations, in March 2011 the CIC hosted its Annual Meeting with Focus on Schedule and Dose for Combination Therapies and in April, the CCIC also reviewed aspects of combination immunotherapy at their 4th annual meeting. Additional meetings were held throughout 2011 with a focus on ways to improve immunotherapy outcomes. In May, CIMT met in Mainz, Germany, for their 9th Annual meeting entitled "Targeting Cancer: Road-Maps for Success". From June 30th until July 1st, The JACI met in Osaka for a symposium on the "Current status and future prospective of cancer immunotherapy". In September, CSCO and SITC hosted a joint cancer immunotherapy session in Xiamen, China, and in October, TIBT met in Jinan, China for their "12th National Tumor Biotherapy Conference" and ESCII and NIBIT joined together in Siena for "New Perspectives in the Immunotherapy of Cancer". Also in October, the PIVAC held their 11th meeting on cancer vaccines in Copenhagen. In November, the SITC hosted their second workshop on the science and logistics of combination therapy [39] and in December, SITC joined with NIBIT and the Italian Melanoma Intergroup in sponsoring "Melanoma research: a bridge from Naples to the World". In 2012 additional meetings focused on cancer immunotherapy are planned. In March the BDA will host their 11th Biological Therapy of Cancer Conference in Munich and TVACT will host their 18th annual meeting on Cancer Immunotherapy in Chicago. In April the CIC will host their annual colloquium outside Washington DC and in May CIMT will host their 10th annual meeting in Mainz. Early in 2012, the European Academy of Tumor Immunology will start writing combinatorial multicentric randomized Phase II trials associating academic GMP vaccines, immunogenic chemotherapy and immune checkpoint

blockade inhibitors so that multiple institutions experienced in immunotherapy and immunomonitoring may be able to conduct this enterprise. While each organization will continue to pursue meetings and activities that address the needs of their members, the consortium of fifteen organizations, termed the World Immunotherapy Council, will work to find areas for collaboration and exchange of scientific information.

5. Limited Funds Available to Translate Science into Patients

Once investigators have identified a novel immunotherapy treatment, with compelling preclinical evidence to support its potential as a treatment for patients with cancer, the challenge of obtaining funding to initiate the clinical trial becomes a rate-limiting barrier. In the USA, reduction in funding by the National Cancer Institute (NCI) has seriously impacted the movement of new treatment strategies to the clinic. The Department of Defense has a number of programs that support translational clinical trials and this has helped fill the gap. The struggling biotech sector provides some help. In the USA some of this is through the NIH-funded Small Business Innovation Research (SBIR) and Small Business Technology Transfer (STTR) programs that have provided needed resources for moving agents to clinical trials. In other instances it is local and state governments, angel investors and philanthropy, more than high risk-adverse venture capital, that support these early phase trials. In the future it is expected that these sources will continue to play an important role in moving innovative first-in-human studies, particularly of cellular and combination immunotherapy studies, to patients with cancer. Investigators in Europe, Canada and Japan are also concerned about limited options to obtain support for translating new immunotherapy strategies to the clinic. However, the Japanese Ministry of Health, Labour and Welfare recently announced a fund of 1.1 billion Japanese yen for cancer vaccine clinical trials over the next 3 years. In China, the new 12th 5 year plan will provide broad support for translational clinical trials. Nonetheless, the majority of

investigators and co-authors consider the difficulty in obtaining funding to initiate clinical trials to be a major hurdle for cancer immunotherapy.

Opportunities:

To effectively communicate the impact investment in translational research and biotechnology/cancer immunotherapy has on the economic development of national and local economies as well as to human health [2].

6. Lack of Definitive Biomarkers of Immune Response

The lack of validated biomarkers for monitoring the development of an immune response following therapy is another critical hurdle for the translation of cancer immunotherapies. The iSBTc-SITC-/NCI/FDA Taskforce for Immunotherapy Biomarkers, composed of nine societies and participating organizations, has addressed this in detail [20,14]. Eight of the nine challenges identified by this Taskforce were related to immunological monitoring considerations. These included issues that should be optimized to obtain validated assays that can provide a reliable platform to compare cancer immunotherapy trials. A ninth challenge related to the identification of biomarkers for cellular immunotherapy products. These issues included:

1. Processing and storage of blood samples to bank peripheral blood mononuclear cells (PBMC) and serum for immunologic studies
2. Characterization of cellular products for therapy
3. Assay standardization and harmonization before testing patient samples
4. Centralization of immunological monitoring
5. Standardized assays that should be used for clinical trial antitumor immune response determination
6. How assay data should be analyzed for "responder" and "non-responder" identification
7. Reporting immunological monitoring data in publications

8. Validation of specific assays and/or analytes as biomarkers of clinical response
9. Novel assays in development for immunological testing of patients

Despite substantial efforts from many groups, immunological monitoring is challenged by two central limitations. First, we do not know which parameters of immune responses are the most important in a clinical response to immunotherapy; secondly, we do not know which assays or sample source (i.e., blood, lymph node, DTH site or tumor) are optimal to assess these parameters and correlate to efficacy. Indeed, the tumor-specific cellular immune response promoted by immunization often has not correlated with clinical cancer regression [40,41]. A contributing reason may be the inherent complexity of immune response assays, in conjunction with variable assay protocols across clinical trial laboratories, which results in high data variability and limited reproducibility [42]. Through more than five years of community-wide proficiency panels on the most commonly used immune response assays (ELISPOT, HLA-peptide multimers, ICS and CFSE) organized by the CIMT and CIC immune monitoring consortia, it could be demonstrated that assay harmonization is an effective mechanism to reduce these limitations [42, 43,44]. Harmonization guidelines resulting from this process are simple to implement, do not impose standardized assay protocols on individual laboratories and improve assay performance without stifling scientific creativity. Assay harmonization may provide a solution for non-validated biomarker assays to minimize data variability and allow correlation of immune monitoring results with clinical outcomes [45].

Another major hurdle in biomarker identification is the low clinical response rates that limit identification of correlates with response to immunotherapies. Indeed, when response rates to immunotherapy reach 50%, it has been possible to identify a significant correlation with objective clinical response in patients maintaining at least 5% tumor-specific T cells in their peripheral blood for at least two weeks [46]. Standardized immune monitoring of large multi-

institution trials has recently allowed for statistically significant correlations of anti-tumor immunity and clinical outcome [47].

Opportunities:

Moving forward, the hurdles specified above will need to be addressed. The report from the iSBTc-SITC/FDA/NCI Taskforce on Immunotherapy Biomarkers [20] builds on the NCI's REMARK criteria [48] as well as other more recent reports, e.g., MIFlowCyt, MIACA, and MIATA [49,50,51]. Integration of standardized procedures and internal controls as well as improved reporting practices will improve the ability to identify immune biomarkers following immunotherapy and other approaches which impact immunity. The group will continue to promote discussion around the importance of standardization and support educational programs aimed at improving the ability to reproducibly assess immunotherapy biomarkers.

7. Conventional Response Criteria May Not Reflect the Patterns of Response to Immunotherapies

RECIST or modified WHO criteria have provided the basis for evaluating whether patients with cancer respond to therapy. These traditional criteria were developed for cytotoxic therapies and evaluate reduction in tumor burden following initiation of treatment. While immune therapies have led to striking and rapid reductions in tumor burdens in some patients, others have experienced progression prior to experiencing tumor regression or have had stabilization of disease. In these latter two instances, patients may ultimately recognize a benefit in overall survival but not be identified as responding to therapy based on conventional response criteria. This pattern of response to therapy has been observed by many investigators but was not systematically captured due to absence of adequate response criteria. In 2004, as part of a collaboration between the iSBTc (now SITC) and the CVC (now CIC) to address issues relevant to the development of cancer immunotherapy, both organizations formed the Cancer Vaccine

Clinical Trial Working Group (CVCTWG), which included participation from the FDA and NCI. CVCTWG held several workshops between 2004 and 2005 with a concluding workshop jointly hosted by CVC and SITC at the 2005 Annual Meetings of both organizations. (http://www.sitcancer.org/meetings/am05/workshop.php). These workshops and the resulting publication with input from more than 180 investigators representing academia, NCI, FDA, and the biotech and pharmaceutical sector, discussed how evaluation of a clinical response to immunotherapy might be modified from that for cytotoxic agents [52].

Following the 2005 meeting, both collaborative and independent efforts of the CIC, CIMT and SITC took place to continue addressing these issues. Involvement from the NCI and FDA was included in many of these discussions. The goal of these meetings was to: a) summarize community knowledge, b) define challenges, and c) offer directions for improvement through community workshops. Resulting knowledge was used to systematically generate and analyze data to arrive at pertinent improvements of conventional clinical endpoints. Four main areas were addressed: 1) CIC and CIMT-CIP immune monitoring proficiency panels including >80 international laboratories across the field defined harmonization criteria to provide quality-control mechanisms and minimize data variability without standardizing laboratory protocols with the ultimate aim to allow for correlation with clinical endpoints [42,43,51,44]. 2) The SITC-FDA Taskforce on Immunotherapy Biomarkers, with input from 9 organizations, addressed the lack of validated biomarkers for monitoring the development of an immune response following therapy and identified 9 challenges critical for the translation of cancer immunotherapies [20] (see section "Lack of Definitive Biomarkers of Immune Response". 3) Clinical patterns of antitumor response for immunotherapeutic agents are more complex than those of chemotherapy [52,53,54,55] and adjustments to RECIST or WHO criteria to capture all patterns should be considered. 4) The translation of an immune response into clinical antitumor activity and possible survival benefit takes time [56,53,54]. Therefore, effects on patient survival may only

be detectable several months after treatment start, which may be reflected in a delayed separation of Kaplan Meier curves. This observation was made as part of a systematic review of publicly available Phase 3 data from cancer immunotherapy trials during a CVC workshop in 2006 [56]. The delayed separation of Kaplan-Meier survival curves may be addressed through revised statistical methods of non-proportional hazards [54,57].

The core aspects of these community recommendations were reviewed at a United States Food and Drug Administration Workshop, which included participation and presentations by both CIC and SITC representatives, and were included in a draft guidance document on "Clinical Considerations for Therapeutic Cancer Vaccines" [58]. This illustrates how the collaborative efforts of community-based organizations can lead to an expansion of immunotherapy clinical trials methodology supporting further advances in the field.

Opportunities:

The discussion on changes to response criteria needs to continue. A recent report used patient outcomes following treatment with ipilimumab, a monoclonal antibody that blocks CTLA-4, to evaluate how proposed new immune-related response criteria (irRC) compared to RECIST or WHO criteria [55]. The important observations from that report were that four patterns of response were all associated with favorable survival.

The four patterns of response to immunotherapy were:

1) shrinkage in baseline lesions, without new lesions;

2) durable stable disease (in some patients followed by a slow, steady decline in total tumor burden);

3) response after an increase in total tumor burden; and

4) response in the presence of new lesions.

The conventional response criteria assumed that early increase in tumor growth and/or development of new lesions indicated progressive disease, which has become synonymous with drug failure. For immunotherapeutic agents, however, initial tumor growth or appearance of new tumors does not necessarily reflect immunotherapy failure nor long-term outcomes and survival. The new irRC more accurately reflect the response patterns associated with immunotherapies, and may permit more comprehensive assessment of cancer immunotherapy clinical trial results as well as provide guidance in the clinical care of patients with cancer receiving immunotherapies. While these new irRC appear promising, prospective evaluation of these criteria following treatment with immune therapy is clearly warranted [57].

The FDA, who actively participated in many of these discussions, agreed that cancer vaccines might require considerable time in order to induce a therapeutic response. To address this the FDA provided specific recommendations for the clinical trial statistical analysis plan in their "Draft Guidance for Therapeutic Cancer Vaccines" [58]. It is important to note that the impact on survival is still the gold standard employed by the US FDA and that is the basis for the recent approval of sipuleucel-T and Yervoy [44,59]. While recent reports of markers of an immune response correlating with outcomes are encouraging, substantial opportunities remain for the development of novel surrogate markers of anti-cancer immunity that correlate with improved survival [47,60].

8. Paucity of Translational Teams of Scientists and Clinicians

While there are centers of excellence with teams of investigators working to translate the latest technologies, there are far too few for the number of diseases that need to be targeted with promising immunotherapies. This needs to be improved. Given the cost for drug development,

industry alone cannot be relied upon to conduct all the early stage testing, particularly since academic translational investigator teams, close to both basic and clinical science, are likely in the best position to move "their" agent into the clinic. This requires an investment in infrastructure. Depending on the class of agent(s) and international setting, this may require simple clean rooms or a complete GMP facility. The necessary infrastructure, however, is not simply bricks and mortar, but human capital as well. Teams including regulatory staff for the substantial protocol and consent development and approval steps, QA/QC support, trained data managers and research nurses, in addition to clinicians and scientists, are required to make this work. Clinicians must be appropriately recognized for the time and energy they spend participating in clinical trials beyond their standard clinical duties (which are often more profitable). A common sentiment is that there is a dramatic shortage of clinicians with a commitment to clinical research. This may be due to health systems that poorly valorize involvement of clinicians in research. Another reason clinicians may not have developed a career path in immunotherapy may be linked to the previous negative experience of cancer immunotherapy. Perhaps the increasing momentum in the field will spark enthusiasm for clinicians to train in this field. Another limitation is the number of PhD scientists that are trained and empowered to move their science to the clinic. Recognition of this, particularly by the Howard Hughes Medical Institute (Med into Grad Initiative) and centers with NIH Clinical and Translational Science Awards (CTSA) has led to development of programs that are successfully targeting incoming PhD students in hopes of developing translational investigators [61]. But having clinical researchers and translational PhD scientists alone is not sufficient. The ability to organize, lead, motivate, meld and sustain multidisciplinary groups of investigator in translational teams is considered a critical hurdle for advancing cancer immunotherapies and has been recently discussed [62]. Recognizing the essential role that team science plays in translational cancer immunotherapy, the SITC, in celebration of their 25th anniversary, developed an award to recognize centers that have excelled in this area and provided a

significant and sustained contribution over the past 25 years [63]. Another signatory organization for this document, the Cancer Research Institute, has been a sustaining source of support for the field of cancer immunology for close to 60 years. Its Pre-doctoral and Post-doctoral Fellowship Programs have trained thousands of immunologists over multiple generations. More recently through its partnership with the Ludwig Institute for Cancer Research, its Cancer Vaccine Collaborative establishes the needed infrastructure, reagent procurement, clinical trials management, and funding to carry out coordinated early-phase clinical trials aimed at developing therapeutic cancer vaccines.

Opportunities:

While the programs noted above provide a basis for training and supporting team science, the majority of Universities do not consider seriously these contributions when evaluating candidates for promotion and tenure. Recognizing the contributions of teams to the advance of translational medicine and human health and developing a structure for evaluating these contributions is an opportunity for this consortium.

9. Need to Enhance Exchange of Information Critical to Advancing the Field

Another component of this "team" hurdle is the exchange of information. Given the increasing complexity it is becoming less feasible for a single group to have the detailed knowledge and resources to investigate, analyze, select and implement the best strategies to move forward in clinical trials for any given indication. A possible solution to this hurdle may be to link clusters of investigators with interest and experience with a given tumor type. The histocompatibility/HLA field might serve as an example for this concept. In that field, participants from around the world supplied reagents, ideas, practical work and shared projects to advance the whole field of transplantation. As a whole, these investigators made progress by helping the entire field through specific input of work and resources, driving significant advances over several decades.

The success of these interactions (workshops, exchanges, central repositories) laid the foundation for bone marrow transplantation and organ transplantation (kidney, heart, liver, lung), all of which would not have been feasible through the efforts of a single individual or organization, or even one regional or national consortium.

Opportunities:

The CITN may be able to promote a similar activity as it brings together multiple groups under the same umbrella. Similarly, societies, primarily those represented by co-authors of this publication, could also play a role in bringing together groups of like-minded investigators. Through its annual meeting, associated programs and other collaborative initiatives, the SITC is committed to facilitating the exchange of information and education among basic and translational researchers, clinicians, and young investigators to advance cancer immunotherapies. Importantly, SITC and the other signatory organizations have initiated a process to join together and develop collaborative projects to catalyze continued success in cancer immunotherapy worldwide. This group, tentatively designated the World Immunotherapy Council, will begin by approaching some of the hurdles addressed in this document, and also by organizing joint scientific meetings and sessions.

Conclusion

The identification of nine critical hurdles (Table 1) is an important beginning for this group of collaborating organizations focused on cancer immunotherapy. In late 2010, representatives of ten organizations met in Washington D.C. to discuss the formation of international working groups that can make recommendations to address these hurdles, facilitate change and improve the translation of novel immunotherapies to patients with cancer. Through this international, collaborative approach—marked by the establishment of the World

Immunotherapy Council—the many investigators and the fifteen organizations involved in this initiative look forward to combining their efforts synergistically to accelerate the delivery of promising new cancer immunotherapies to patients around the world.

Competing interests

BAF – Co-Founder UbiVac, SAB Micromet, SAB MannKind; PAA - participated in advisory board for Bristol Myers Squibb, GSK, Schering-Plough/Merck and Roche. He has received honoraria from Bristol Myers Squibb and Schering-Plough/Merck; NLB - employee of IRX Therapeutics, scientific advisor for Immunovaccine Technologies and Roche Canada, stock options for sanofi Aventis; CMB is an employee of Ribological GmbH; JAG - Employee of Alnylam **Pharmaceuticals**; KH - employee and stockholder of Celgene Corporation; RBH - Employee of Intrexon Corporation; AH - Employee Bristol-Myers Squibb; SJ – Founder and president of ZellNet Consulting; HIL – Employee of Roche; J-PM - Employee of Merck KGaA; HS-J - Co-founder and employee of Immatics Biotechnologies GmbH; WS - Employee of Takeda Pharmaceuticals; JMW - Employee Bristol-Myers Squibb; All other authors – No competing interests;

Authors' contributions

BF prepared the manuscript collaboratively with input and review by all co-authors representing their respective organizations. All authors have read and approved the final manuscript.

Consent

All individuals within the figures gave informed consent for publication of their image.

Acknowledgements

This manuscript is the output of collaborative summits that were organized and hosted by the Society for Immunotherapy of Cancer (SITC) at their 24th and 25th Annual Meetings. BAF acknowledges support of Earle M. Chiles and The Chiles Foundation, Robert W. Franz, Elsie

Franz Finley, Lynn and Jack Loacker, Wes and Nancy Lematta, the Safeway Foundation, and the Providence Portland Medical Foundation. The work of CIMT-CIP was supported by a grant from the Wallace Coulter foundation (Florida, USA). The authors wish to acknowledge the following organizations, whose representatives contributed to this manuscript:

- Society for Immunotherapy of Cancer (SITC; formerly the International Society for Biological Therapy of Cancer, iSBTc)
- Association for Cancer Immunotherapy Immunoguiding Program (CIMT-CIP)
- Biotherapy Development Association (BDA)
- Canadian Cancer Immunotherapy Consortium (CCIC)
- Cancer Immunotherapy Consortium (CIC) of the Cancer Research Institute (CRI)
- Chinese Society for Clinical Oncology (CSCO)
- Committee for Tumor Immunology and Bio-therapy (TIBT)
- Dutch Tumor Immunology Working Party (DTIWP)
- European Academy of Tumor Immunology (EATI)
- European Society for Cancer Immunology and Immunotherapy (ESCII)
- Italian Network for Tumor Biotherapy (NIBIT)
- Japanese Association of Cancer Immunology (JACI)
- Nordic Center for Development of Antitumour Vaccines (NCV-network)
- Progress in Vaccination Against Cancer (PIVAC)
- Tumor Vaccine and Cell Therapy Working Group (TVACT)

Table 1. Critical Hurdles in Cancer Immunotherapy Identified by SITC and Collaborating Associations

1. Limitations of current animal models to predict efficacy of cancer immunotherapy strategies in humans
2. Prolonged time to obtain approval to initiate clinical trials
3. Complexity of cancer, tumor heterogeneity and immune escape
4. Limited availability of reagents for combination immunotherapy studies
5. Limited funds available to translate science into patients
6. Lack of definitive biomarker(s) for assessment of clinical efficacy of cancer immunotherapies
7. Conventional clinical response criteria do not take into consideration differences between response patterns to cytotoxic agents and immunotherapies
8. Paucity of teams of scientists and clinicians dedicated to translational research in cancer immunotherapy
9. Insufficient exchange of information critical to advancing the field

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Figure Legends:

Figure 1: 2009 Immunotherapy Summit at SITC creating the working group, National Harbor, MD, USA
Back row: Leif Haakason, Sylvia Janetski, Franco Marincola, Lisa Butterfield, Hideaki Tahara, Dolores Schendel, F Stephen Hodi, Heinz Zwierzina, A. Raja Choudhury, Graham Pawlec, Wenru Song.
Front row: Tom Gajewski, Bernard A. Fox, Mary Disis, Michael Papamichail, Michael B. Atkins

Figure 2: 2010 Immunotherapy Summit at SITC, Capital Hill, Washington DC, USA
Back row: Michael Papamichail, Hideaki Tahara, Howard Kaufman, Jedd Wolchok, Franco Marincola, James Finke, Rejean Lapointe, Hyam I. Levitsky, George Coukos, Wenru Song, Padmanee Sharma, F Stephen Hodi, Jim Allison, Lisa Butterfield, William Murphy, Leif Haakson, A. Raja Choudhary, Heinz Zwierzina, Yutaka Kawakami, Kohzoh Imai. Front row: Harpreet Singh-Jasuja, Michele Maio, Paolo Ascierto, Giorgio Parmiani, Bernard A. Fox, Axel Hoos, Tom Gajewski, Dolores Schendel, Cedrik Britten.

Figure 3: 2011 Immunotherapy Summit at SITC, North Bethesda, MD, USA
Back row: Michele Maio, Michael Papamichail, Michael Nishimura, Bernard A. Fox, Andrea Nicolini, Jens-Peter Marschner, Tanja de Gruijl, Brad Nelson, Axel Hoos, Tetsuro Sasada, Yutaka Kawakami, Rejean Lapointe, Christoph Huber, Jonathan L. Bramson, Pawel Kalinski, Paolo Ascierto, Giuseppe Masucci, Heinz Zwierzina, Franco Marincola, F Stephen Hodi, Per Thor Straten, Jianda Yuan, Front row: Samir Khleif, Lisa Butterfield, Tom Gajewski, Graham Pawlec, Pam Ohashi, Cornelius Melief, Cedrik Britten.

Published OnlineFirst July 6, 2011; DOI:10.1158/0008-5472.CAN-11-1076

Integrated Systems and Technologies

Cancer Research

Genome-wide Profiling of Chromatin Signatures Reveals Epigenetic Regulation of MicroRNA Genes in Colorectal Cancer

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Abstract

Altered expression of microRNAs (miRNA) occurs commonly in human cancer, but the mechanisms are generally poorly understood. In this study, we examined the contribution of epigenetic mechanisms to miRNA dysregulation in colorectal cancer by carrying out high-resolution ChIP-seq. Specifically, we conducted genome-wide profiling of trimethylated histone H3 lysine 4 (H3K4me3), trimethylated histone H3 lysine 27 (H3K27me3), and dimethylated histone H3 lysine 79 (H3K79me2) in colorectal cancer cell lines. Combining miRNA expression profiles with chromatin signatures enabled us to predict the active promoters of 233 miRNAs encoded in 174 putative primary transcription units. By then comparing miRNA expression and histone modification before and after DNA demethylation, we identified 47 miRNAs encoded in 37 primary transcription units as potential targets of epigenetic silencing. The promoters of 22 transcription units were associated with CpG islands (CGI), all of which were hypermethylated in colorectal cancer cells. DNA demethylation led to increased H3K4me3 marking at silenced miRNA genes, whereas no restoration of H3K79me2 was detected in CGI-methylated miRNA genes. DNA demethylation also led to upregulation of H3K4me3 and H3K27me3 in a number of CGI-methylated miRNA genes. Among the miRNAs we found to be dysregulated, many of which are implicated in human cancer, miR-1-1 was methylated frequently in early and advanced colorectal cancer in which it may act as a tumor suppressor. Our findings offer insight into the association between chromatin signatures and miRNA dysregulation in cancer, and they also suggest that miRNA reexpression may contribute to the effects of epigenetic therapy. *Cancer Res; 71(17); 5646–58. ©2011 AACR.*

Introduction

MicroRNAs (miRNA) are a class of small noncoding RNAs that regulate gene expression by inducing translational inhibition or direct degradation of target mRNAs through base pairing to partially complementary sites (1). miRNA genes are transcribed as large precursor RNAs, called pri-miRNAs, which may encode multiple miRNAs in a polycistronic arrangement. The pri-miRNAs are then processed by the RNase III enzyme Drosha and its cofactor Patha to produce approximately 70-nucleotide hairpin structured second precursors (pre-miRNAs). The pre-miRNAs are then transported to the cytoplasm and processed by another RNase III enzyme, Dicer, to generate mature miRNA products. miRNAs are highly conserved among species and play critical roles in a variety of biological processes, including development, differentiation, cell proliferation, and apoptosis. Subsets of miRNAs are thought to act as tumor suppressor genes (TSG) or oncogenes, and their dysregulation is a common feature of human cancers (2). More specifically, expression of miRNAs is generally downregulated in tumor tissues, as compared with the corresponding normal tissues, which suggests that some miRNAs may behave as TSGs in some tumors. Although the mechanism underlying the alteration of miRNA expression in cancer is still not fully understood, recent studies have shown that multiple mechanisms involved in regulating miRNA levels are affected in cancer. For example, genetic mutations that affect proteins involved in the processing and maturation of miRNA can lead to overall reductions in miRNA expression levels (3, 4). In addition, genetic and epigenetic alterations can disrupt expression of specific miRNAs in cancer.

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Note: Supplementary data for this article are available at Cancer Research Online (http://cancerres.aacrjournals.org/).

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doi: 10.1158/0008-5472.CAN-11-1076