

Wound Healing Metabolites to Heal Cancer and unhealed wounds

Abstract

Cytotoxic agents were the choice of cancer establishments to combat cancer when President Nixon declared War on Cancer in 1971. After the failure to win the war on cancer during the 5 years of intensive presidential support, it was concluded that cytotoxic agents were unable to win the war on cancer. The emphasis of cancer research was then shifted from cytotoxic agents to DNA research, and gene and targeted therapies during the period of 1976 – 1995. Entire human genomes were sequenced which was a phenomenal achievement. The achievement, however, helped very little on cancer therapy. Studies of aberrant DNA methylations became a fashion, which, however, failed to grasp the critical issue of abnormal methylation enzymes to let the solution of cancer to slip away. Gene therapy was too difficult and too expensive to yield acceptable cancer drugs. Many excellent targeted drugs were discovered, which were good differentiation helper inducers to promote terminal differentiation of cancer cells. These excellent cancer drugs could not replace cytotoxic agents because they were unable to cause the tumor to disappear. These excellent targeted cancer drugs were primarily used for the therapy of hematological cancers. The emphasis was then shifted to anti-angiogenesis studies during 1995-2015, which did not produce good cancer drugs, and now to the immunotherapy, which has produced promising drugs for lung cancer. Immunotherapy has the potential to replace cytotoxic agents. Immunotherapy, however, appears to have the same problems as cytotoxic agents to cause damage to chemo-surveillance and to show ineffectiveness against cancer stem cells, which were primarily responsible for the failure of cytotoxic agents to win the war on cancer. Such deleterious effects can be remedied by the employment of cell differentiation agent formulations.

Wound healing metabolites are the nature's creation of chemo-surveillance to ensure perfection of wound healing to avoid cancer evolution. Cancer arises due to the collapse of chemo-surveillance, thus, wound healing metabolites are the right medicines to heal cancer. These drugs may also be applicable for the therapy of diseases arising due to the collapse of chemo-surveillance such as dementia and tissue fibrosis.

Running title: Wound healing metabolites to heal cancer

Key words: abnormal methylation enzymes; cachexia; cancer stem cells; cell differentiation; chemo-surveillance; progenitor stem cells; wound healing.

Abbreviations used are: AA, arachidonic acid; AdoHcy, adenosylhomocysteine; AdoMet, adenosylmethionine; AFB₁, aflatoxin B₁; A10, antineoplaston A10; CCs,

cancer cells; CDA, cell differentiation agent; CSCs, cancer stem cells; DHIs, differentiation helper inducers; DIs, differentiation inducers; MAT, Methionine adenosyltransferase; MDS, myelodysplastic syndrome; MEs, methylation enzymes; MT, methyltransferase; PGs, prostaglandins; PSCs, progenitor stem cells; RI_{0.5}, reductive index_{0.5}; SAHH, S-adenosylhomocysteinehydrolase; TET-1, ten-eleven translocation-1; TNF, tumor necrosis factor.

Introduction

Cytotoxic chemotherapy of cancer was a tragic byproduct of World War II. Sulfur mustard gas bombs were used during the war. Victims died of poisonous gas all displayed deficiency of lymphocytes, which inspired oncologists to employ toxic chemicals to treat leukemia patients. Oncologists were apparently satisfied with the selective toxicity of cytotoxic agents toward leukemia cells. Cytotoxic agents quickly became the standard therapeutic agents not only for leukemia, but also for solid tumors. Disappearance of leukemia cells and tumor became the standard criterion for the evaluation of therapeutic efficacy. When President Nixon declared "War on Cancer" in 1971, cytotoxic agents were the choice of cancer establishments to combat cancer. Cancer establishments, however, failed the challenge to win the war on cancer during the five years of intensive presidential support. If a treatment modality has been drilled through as a presidential project and failed to achieve the goal to win the war on cancer, it was fair to conclude that this treatment modality was unable to win the war on cancer. Apparently, cancer establishments agreed on this conclusion to shift the emphasis of cancer research from cytotoxic agents to DNA research, and gene and targeted therapies. Entire human genomes were sequenced, which was a phenomenal achievement. The achievement, however, helped very little on cancer therapy. Studies of DNA methylation became a fashion around 1985, disclosing a lot of aberrant DNA methylations of cancer cells. Studies of tRNA methylation were also a fashion around 1966 finding a lot of aberrant tRNA methylations. Cancer establishments were brilliant to identify the important issues of cancer, but unfortunately failed to grasp the most critical issue of abnormal Methylation Enzymes (MEs) to let the solution of cancer to slip away around 1966 and 1985.

Emphasis on DNA research during 1976-1995 did not produce cancer drugs that could replace cytotoxic agents. Gene therapy was a fascinating field. But the technology was simply too difficult and too expensive to yield acceptable drugs. Studies of targeted therapies on inhibitors of growth factors and signal transductions did produce many excellent cancer drugs. These inhibitors were good Differentiation Helper Inducers (DHIs) [1]. The therapeutic endpoint of DHIs was the terminal differentiation of Cancer Cells (CCs) which was unable to cause the shrinkage of tumor. These excellent cancer drugs were mostly used in the therapy of

hematological cancers. The criterion of tumor disappearance prevented the discovery of good cancer drugs not based on cell killing.

Since DNA research did not produce cancer drugs that could compete with cytotoxic agents to cause complete shrinkage of tumor, the emphasis was then shifted to anti-angiogenesis during 1995-2015, only found anti-angiogenesis agents to cause more deaths due to bleeding than cytotoxic agents due to toxicity and ineffectiveness against Cancer Stem Cells (CSCs). After failure of anti-angiogenesis attempt, the attention was then shifted to immunotherapy, which did produce encouraging drugs on the therapy of lung cancer. We don't know if it can replace cytotoxic therapy. It is definitely more selective than cytotoxic agents to take out CCs. But it has the same problems as cytotoxic agents to cause damage to chemo-surveillance and may not be effective against CSCs. CSCs are PSCs minus TET-1 [2]. The antigenicity of CSCs should be the same as PSCs, which is tolerable to the human immune system. Still immunotherapy has the advantage not to harm normal stem cells. The deleterious effects to cause the damage to chemo-surveillance and to show ineffectiveness against CSCs can be remedied by the application of Cell Differentiation Agent (CDA) formulations [3].

Our studies strongly suggested that cancer arose as a consequence of wound not healing properly [3]. The concept of cancer as a non-healing wound was first introduced by a great German scientist Virchow in 19th century [4]. It was again brought up by Dvorak in 1986 [5]. We provided the most important details on this subject that included abnormal MEs to block differentiation [6-8]; Differentiation Inducers (DIs) and DHIs as wound healing metabolites and also as the active players of chemo-surveillance [9-11]; hypomethylation of nucleic acids as the most critical mechanism to accomplish terminal differentiation of PSCs [12]; the evolution CSCs from Progenitor Stem Cells (PSCs) due to the collapse of chemo-surveillance [9]; and the mechanism of wound healing and the impact of wound on the evolution of cancer [3, 13-15]. Since cancer is caused by a wound not healing properly, wound healing metabolites must be the most appropriate medicines for cancer therapy [16, 17].

Commentaries and Discussions

Wound Healing and the Evolution of Cancer

Wound healing and the evolution of cancer are closely related to involve PSCs as the critical common elements. Wound healing is a process to involve the breakdown of membrane bound phosphatidyl inositol to release arachidonic acid (AA) for the synthesis of prostaglandins (PGs) [18], which are responsible for the initial stage of wound to promote the proliferation of PSCs. The final stage of wound healing is carried out by the wound healing metabolites, DIs and DHIs, to promote the terminal differentiation of PSCs. DIs and DHIs are the important constituents of chemo-surveillance [9-11]. Healthy persons are able to maintain a steady level of DIs and DHIs to direct efficient terminal differentiation of PSCs to heal the wound. So wound healing comes naturally to healthy persons without having to put up any effort. Sutures and antibiotics are

subsidiary of wound healing to speed up and to prevent infection. But if DIs and DHIs are not sufficient due to pathological conditions, then the wound healing process will be affected to allow PSCs to evolve into CSCs. It takes only a single hit to silence TET-1 enzyme to convert PSCs to CSCs, which is very easy for PSCs to accomplish because these cells have abnormally active MEslike CCs [6-8]. Chemo-surveillance is the nature's creation to prevent that from happening. The protection of the functionality of chemo-surveillance is very important to ward off cancer. Wound also triggers immunological response to produce cytokines. Tumor Necrosis Factor (TNF) among such cytokines is bad for wound healing. TNF is also named cachectin after its effect to induce cachexia symptom. TNF is toxic to proliferating cells to induce apoptosis, normal stem cells and CCs included. It is also active to induce hyperpermeability of blood vessel [19, 20] to cause excessive excretion of low molecular weight metabolites. Wound healing metabolites are among such low molecular weight metabolites excreted. The consequence is the loss of ability to heal the wound, thus allowing PSCs to evolve into CSCs, and then to progress to faster growing CCs by the activation of oncogenes and/or the inactivation of suppressor genes.

Chemo-surveillance

Chemo-surveillance was a term we created to describe a natural defense mechanism against Cancer [9]. Now, we modify it as a term to ensure perfection of wound healing as the primary objective and the defense of cancer as the secondary consequence [10, 11]. Whatever comes naturally is the nature's creation to benefit human beings. The prime example is the photosynthesis that turns CO₂ into O₂. Immuno-surveillance is another example that is well accepted. Chemo-surveillance we brought up was completely ignored because the active elements DIs and DHIs were non-toxic unacceptable to the concept of destruction of cancer with toxic agents. We used peptides as the surrogate molecules of wound healing metabolites to carry out studies of chemo-surveillance. The plasma and urinary peptide analyses of cancer patients as shown in the following Table 1 clearly shows that cancer patients excrete excessive amounts of peptides resulting in the decrease of plasma/urine ratios.

Table 1. Plasma/urine peptide ratios of cancer patients

Plasma/Urine CDA Ratios	Number of Patients	Number of % Distribution	
0.8-0.83 (normal)	5	2	1.8
0.6-0.8	4	7	6.5

0.4-0.6	3	18	16.7
0.2 -0.4	2	38	35.2
0.1 - 0.2	1	24	22.2
0.02- 0.1	0	19	17.6

Table 1 is reproduced from the data published in the reference [9]. The column of CDA Scores is a new addition. Peptide analyses were conducted as previously described [9] by purification of peptides through C18 cartridge, and then ran peptide analysis by HPLC resolution and Ninhydrin reaction. The unit of plasma peptides was nmole/ml, and the unit of urinary peptides was nmole/mg creatinine.

Plasma/urine peptide ratios correspond very well to the severity of cancer patients. Antineoplastons are urinary wound healing metabolites purified by C18 reverse phase chromatography as the purification of peptides above described. If cancer patients responded well to the therapy with antineoplastons, their plasma/urine peptide ratios would increase and eventually reached the level of healthy persons [21]. The therapy with antineoplaston A10, the code name for phenylacetylglutamine, produced similar results [9]. The favorable responses to antineoplaston A10 were limited to patients with CDA scores of 3 and above. Antineoplaston A10 was inactive as DI nor as DHI. It did not have inhibitory effect on HL-60 cells even at a very high concentration of 100 mM, but it had remarkable effect to prevent hepatocarcinogenesis induced by potent hepatocarcinogen Aflatoxin B₁ (AFB₁) as shown in Figure 1. By keeping the functionality of chemo-surveillance intact, wound healing metabolites, namely CDA components, could effectively prevent the hepatocarcinogenesis induced by AFB₁. Our studies clearly indicate that chemo-surveillance is a very effective mechanism to ensure perfection of wound healing. If a wound can be efficiently healed, then cancer evolution can be avoided [9-11]. The protection of the functionality of chemo-surveillance is very important for the efficient wound healing. PGs produced in response to wound is helpful for the maintenance of chemo-surveillance and wound healing. On the contrary, TNF produced in response to wound is bad for wound healing due to its effect to induce cachexia symptom. This bad effect can be effectively antagonized by A10, namely phenylacetylglutamine.

Figure 1. Protective effect of antineoplaston A10 on hepatocarcinogenesis Induced by aflatoxin B₁

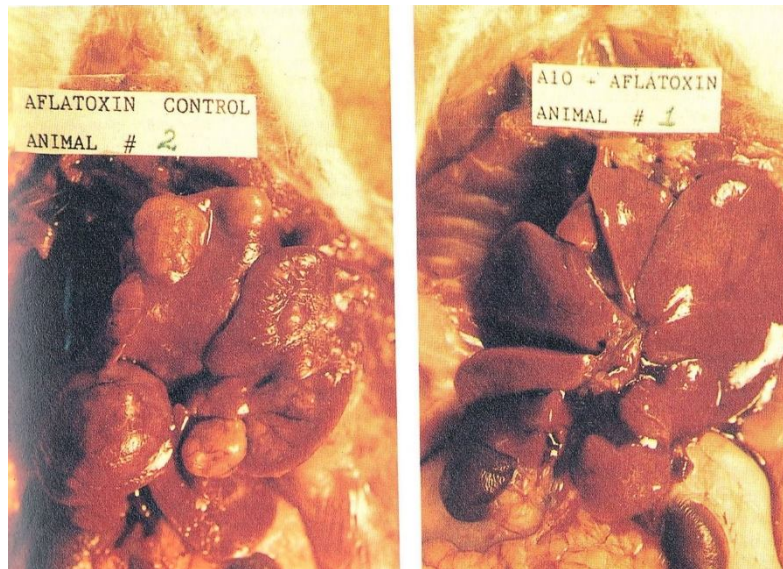


Figure 1 is reproduced from the data published in the reference [22]. Male Fisher rats, 26 each group, were fed control diet or diet with 1% A10. AFB₁ dosing was started 8 days after feeding with A10 diet. AFB₁ was administered by gavage at the dose of 25 µg/day, 5 days weekly for 8 consecutive weeks. Animal #1 was the rat fed A10 showing liver without neoplastic lesion. Animal #2 was the rat fed diet without A10 showing numerous lesions.

Myelodysplastic syndrome (MDS) is a classic disease to illustrate the evolution of cancer due to wound not healing properly. MDS often starts with a display of an immunological disorder [23], which prompts the production of inflammatory cytokines. Among such cytokines, TNF is the critical factor related to the development of MDS, because antibody of TNF could effectively reverse the progression of the disease at the early stage [24]. The propagating cells of MDS have been identified as a rare form of human CSCs [25]. Therefore, MDS is at a stage of CSCs evolved from PSCs. During our studies of chemical hepatocarcinogenesis, we were able to detect abnormal MEs in the preneoplastic hyperplastic nodules, which might represent the proliferation of PSCs [26]. Therefore, the genesis of cancer must proceed from PSCs to CSCs, and then progress to CCs.

Abnormal Methylation Enzymes as the Bullseye of Cancer Target

Had the cancer establishments focused the attention on abnormal MEs during the fashionable studies of aberrant tRNA methylations around 1966 and aberrant DNA methylations around 1985, cancer might have been solved. In 1966, before the declaration of War on Cancer, and in 1985 after the declaration of War on Cancer that failed. Cancer establishments missed the critical issue of abnormal MEs to win the war on cancer [27].

MEs play a critical role on the regulation of cell replication, differentiation and apoptosis by virtue of the fact that DNA MEs control the expression of tissue-specific genes [28], and pre-

rRNA MEs control the production of ribosome [29], which in turn dictates the commitment of cells to initiate replication [30]. If enhanced production of ribosome is locked in place, it becomes a factor to drive carcinogenesis [31]. Biological methylation is mediated by a ternary enzyme complex consisting of Methionine Adenosyltransferase (MAT)-Methyltransferase (MT)-S-AdenosylhomocysteineHydrolase (SAHH) [32, 33]. MEs must be in the ternary enzyme complex to become stable and functional. SAHH is the most unstable enzyme of the three MEs which requires a stabilizing factor to protect its stability. Steroid hormones are the stabilizing factors of SAHH of the steroid hormone target tissues. Other tissues may require factors similar to steroid hormones to protect the stability of SAHH. These stabilizing factors of SAHH are often the important factors for the regulation of MEs to influence cellular functions.

MEs become associated with telomerase in cell expressing telomerase. The association with telomerase changes kinetic properties and the regulatory functions of MEs. The K_m values of the telomerase associated MAT-SAHH isozyme pair are 7-fold higher than the K_m values of the normal isozyme pair. The increased K_m values offer greater stability of the abnormal MEs associated with telomerase. It has been shown by Prudova et al [34].³⁴ that the binding of S-adenosylmethionine (AdoMet) greatly increased the stability of protein against protease digestion. The increased K_m values expand pool sizes of AdoMet and S-adenosylhomocysteine (Ado-Hcy). A bigger pool size of AdoMet and AdoHcy is obviously required to maintain malignant growth. It was shown by Chiba et al [35], that the induction of terminal differentiation of HL-60 cells resulted in great shrinkage of the pool sizes of AdoMet and AdoHcy. These studies support our findings that abnormal MEs play an important role to promote malignant growth. Since abnormal MEs play such an important role to promote malignant growth, destabilization of abnormal MEs by DIs and DHIs is an effective strategy to combat cancer [33, 36, 37]. DIs are chemicals capable of eliminating telomerase from abnormal MEs, and DHIs are inhibitors of MEs which can greatly potentiate the activity of DIs. DIs and DHIs are actually wound healing metabolites of the nature's creation to play the role of chemo-surveillance. The strategy of destabilization of abnormal MEs is a perfect cancer therapy that restores the functionality of chemo-surveillance to promote terminal differentiation of both CCs and CSCs. By promoting terminal differentiation of both CCs and CSCs, wound healing metabolites can also put to rest gene abnormalities that contribute to malignant growth. Oncogenes and suppressor genes are cell cycle regulatory genes. They have important role to play when cells are in cell cycle replicating. But if replicating cells exit cell cycle to undergo terminal differentiation, they have no role to play. So, induction of terminal differentiation is an easy way to solve gene abnormalities which are otherwise very difficult to solve. Wound healing metabolites have a unique advantage no other cancer drugs can compete. They are able to take out PSCs and CSCs protected by drug resistance and anti-apoptosis mechanisms [36]. Repair is the biological mission of these cells. Wound healing metabolites are the partner to their biological mission. Therefore, wound healing metabolites can easily access these cells to promote terminal differentiation of PSCs and CSCs to accomplish healing role. A complete remission achieved by wound healing metabolites is worth life time, whereas recurrence is a common happening to other therapies.

CDA-2 as a Perfect Cancer Drug

CDA-2 is a preparation of wound healing metabolites purified by reverse phase

chromatography using XAD-16 as the adsorbant and ethanol as the organic solvent [38]. The active components include AA as the major DI, and pregnenolone and uroerythrin as the major DHIs [2, 38]. Phenylacetylglutamine is a major chemical component as an anti-cachexia agent. The therapeutic endpoint is the induction of terminal differentiation as shown in Figure 2. It could greatly improve the quality of life of patients undergoing cytotoxic chemotherapy, but could not cause the tumor to shrink. It was approved by the Chinese FDA for cancer therapy as a supplement to chemotherapy in 2004 [39]. CSCs was not an issue when the clinical trial of CDA-2 was conducted. The effect of CDA-2 on CSCs was not evaluated. The ability of CDA-2 to take out CSCs, and to restore the functionality of chemo-surveillance was a great improvement to cytotoxic chemotherapy.

Figure 2. Effectiveness of CDA-2 on the induction of terminal differentiation of human Smmn7722 hepatocellular carcinoma xenografted into nude mice

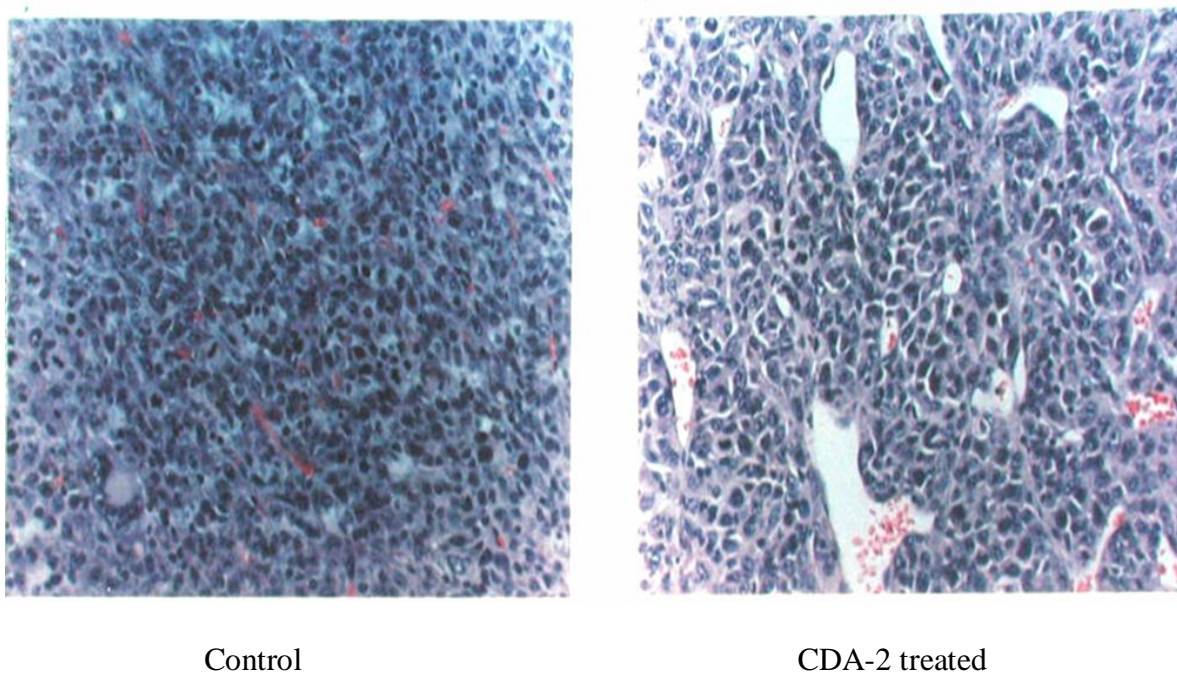


Figure 2 is reproduced from the data published in the reference [36]. Smmn7722 hepatocellular carcinoma cells were xenografted subcutaneously into nude mice. The tumors were allowed to grow to the sizes of around 1 cm diameter, and then started to treat with CDA-2. CDA-2 was administered by IP injection of 1 ml of 100 mg/ml CDA-2 injection fluid/mouse/day, 5 days a week for one month. Control mice received IP injection of 1 ml of saline solution. At the end of one month, mice were sacrificed, and tumors excised to fix for making slide sections, which were stained with trypan blue staining solution, and examined under microscope. CD-2 treated tissue showed well organized differentiation structure similar to hepatic tissue, whereas the control tissue showed totally disorganized structure.

Actually, CDA-2 is best for the therapy of MDS, a disease attributable entirely to CSCs. The therapy requires the differentiation of pathological CSCs to become functional cells. The clinical trial of CDA-2 on MDS was conducted during 2004 to 2007 when Ming C. Liu was in charge of

clinical development of CDA-2 for the Ever Life Pharmaceutical Company which manufactured CDA-2. Dr. Jun Ma, the Director of Harbin Institute of Hematology and Oncology who was then the chairman of Chinese Society of Clinical Oncology, carried out clinical trial of CDA-2 on 117 MDS patients. Based on two cycles of treatments, each 14 days, the therapeutic efficacy of CDA-2 in comparison to Vidaza and Decitabine as shown in Figure 3 was slightly better based on cytological evaluation, and markedly better based on hematological improvement evaluation, namely becoming independence on blood transfusion. Better yet, CDA-2 was totally devoid of serious adverse effects, whereas Vidaza and Decitabine were proven carcinogens [40, 41] and very toxic to DNA [42-44]. CDA-2 is definitely a better drug than Vidaza and Decitabine for the therapy of MDS. CDA-2 was approved for the therapy of MDS by the Chinese FDA in 2017 [36].

Figure 3. Effectiveness of CDA-2 for the therapy of MDS in comparison to Vidaza and Decitabine

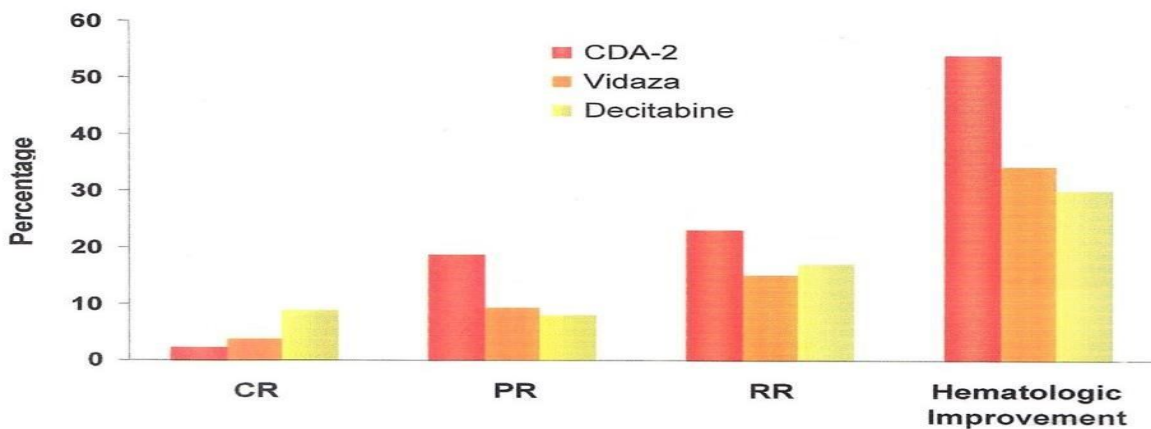


Figure 3 is reproduced from the data published in the reference [36]. MDS patients received daily injection of 2 bottles of 100 ml CDA-2 injection, 100 mg/ml, diluted with one liter isotonic glucose solution through catheter enclosed in the chest. A patient received two cycles of CDA-2 injection, each 14 days. In between there was a period of 7 days rest for evaluation. Patients receiving Vidaza and Decitabine were historical cases treated at Harbin Institute of Hematology and Oncology, who were treated with optimal dosages of Vidaza and Decitabine under the similar protocol. CR stands for complete remission, PR for partial remission, and RR for the two combined. Hematologic improvement is a measure of dependence on blood transfusion to stay alive.

Cancer is caused by multiple factors including the breakdown of chemo-surveillance due to TNF [9, 23], evolution of CSCs from PSCs due to the failure of wound healing, abnormal MEs to

block differentiation and activation of oncogenes and/or inactivation of suppressor genes. Phenylacetylglutamine of CDA-2 can antagonize TNF. DI and DHIs can take care of differentiation blockade of both CCs and CSCs. By promotion of terminal differentiation, CDA-2 can also put to rest gene abnormalities. Therefore, CDA-2 is a perfect cancer drug to eliminate all important factors contributing to the development of cancer.

DIs and DHIs for the Design of CDA Formulations

We have discovered many effective DIs and DHIs for the design of CDA formulations, which are listed in Tables 2 and 3. DIs are listed as ED₂₅, ED₅₀ and ED₇₅, and DHIs are listed as reductive index_{0.5}, which is an expression of potency. The dosage of a DHI to achieve a reductive index_{0.5} is equivalent to the dosage of a DI to achieve ED₂₅. These data have been previously published [1, 37, 38, 45-47].

Table 2. Effective DIs We Have Studied, Most of Which were Our Discoveries

DIs	ED ₂₅	ED ₅₀	ED ₇₅
Phorbalester, nM	0.17	0.26	0.38
Retinoic acid, μM		0.18	0.36
PGI ₂ , μM		7.9	13.8
16, 16-dimethylPGE ₂ , μM		10.8	17.3
PGE ₂ , μM		20.6	32.0
BicycloPGE ₂ , μM		21.0	43.5
AA, μM		24.0	46.8
BIBR1532, μM		32.3	43.7
Boldine, μM		60.1	78.3

Table 2 is reproduced from the data published in the references [46, 47]. Phorbalester and retinoic acid were well known DIs discovered by others. AA and PGs derivatives were wound healing metabolites, and BIBR1532 and boldine were telomerase inhibitors.

Table 3. Inhibitors of MEs as DHIs

SAHH Inhibitors	RI _{0.5} , μM	MT Inhibitors	RI _{0.5} , μM
Pyrvinium pamoate	0.012	Ethidium bromide	1.10
Vitamin D3	0.61	Uroerythrin	1.75
Dexamethasone	0.75	Hycanthone	2.10
Testosterone	1.55	Riboflavin	2.30
Gugulsterone	1.59		
Beta-Sitosterol	1.72		
Dehydroepiandrosterone	1.79	MAT Inhibitors	RI _{0.5} , μM
Dihydrotestosterone	2.10		
Prenisolone	2.22		
Estradiol	2.45	Indolacetic acid	220
Progesterone	3.55	Phenylacetylvaline	500
Hydrocortisone	4.59	Phenylacetylleucine	780
Pregnenolone	7.17	Phenylacetylisoleucine	800
Pregnenolonesulfate	7.35	Butyric acid	850
Phenylbutyric acid	970		

Table 3 is reproduced from the data published in the references [1, 38]. All DHIs were our discovery. The potency of DHI RI_{0.5} was determined as previously described [45]. The dosage of DHI to achieve RI_{0.5} is equivalent to DI of ED₂₅.

Table 4. Inhibitors of Signal Transductions and Growth Factors as DHIs

Signal Transduction Inhibitors	RI _{0.5} , μM	Growth Inhibitors	RI _{0.5} , μM
Sutent	0.28	Arsenic acid	0.28
Berberine	1.62	Cobalt chloride	0.62
Vorient	10.1	Selenite	19.7
Gleevec	11.9		
Metformin	44.9		

Table 4 is reproduced from the data published in the reference [1]. These DHIs were our discovery

Table 5. Polyphenols as DHIs

Polyphenols RI _{0.5} , μM	Polyphenols RI _{0.5} , μM		
Tannic acid	0.37	Pyrogallol	3.18
Epigallocatechin gallet	0.62	Silibinin	3.30
Resveratrol	1.16	Caffeic acid	3.87
Curcumin	1.24	Ellagic acid	4.45
Kuromanin 1.43	Gallic acid	5.35	
Coumestrol 1.95	Ferulic acid	7.41	
Genisteine 2.16	Phloroglucinol	38.8	
Pterostilbene 2.19			

Table 5 is reproduced from the data published in the reference [1]. These DHIs were our discovery.

From the active DIs and DHIs listed in Tale 2 and Table 3 through 5, it is easy to design CDA formulations to accomplish the induction of terminal differentiation to reach 100%. DIs alone cannot reach 100%, so ED₇₅ is about the maximal dosage of DIs, and if supplemented with DHI of RI_{0.5} could achieve the induction of terminal differentiation to reach 100%. Those dosages are in the amounts per liter of blood. A normal person has 5 liters of blood. We have to multiply the amounts by a factor of 5 to provide enough medicines to reach 100% terminal differentiation of cancer cells. Three times a day of the maximum dosages to achieve 100% induction of terminal differentiation of cancer cells should provide good therapy of cancer.

The employment of wound healing metabolites for cancer therapy offers a unique advantage to eliminate CSCs, which is very important for the completion of cancer therapy. We were aware that the winner of the contest to eliminate CSCs won the contest of cancer Therapies [48]. In consideration of designing CDA formulations for specific cancer, we have to pay attention to specific problem confronting that particular cancer. For example, brain cancer has the problem of blood brain barrier, melanoma has the problem of hypoxia, and pancreatic cancer has the problem of collagen envelope. So, there are issues not related to cancer to contribute to cancer problems.

Therapeutic endpoint of CDA formulations is the induction of terminal differentiation. The evaluation of therapeutic efficacy must be set differently from the disappearance of tumor set for cytotoxic agents. Disappearance of cancer markers or circulating CCs and CSCs may be the valid endpoints for the evaluation of CDA formulations on cancer therapy. The elevation of CDA

score to the 5 of healthy persons as listed in the Table 1 may be helpful for the evaluation of therapeutic efficacy of CDA formulations.

Apparently, wound healing metabolites are the right drugs for the therapy of cancer which is the most feared disease arising due to the collapse of chemo-surveillance. Untreatable diseases arising due to the collapse of chemo-surveillance may include dementia [49] and tissue fibrosis [50, 51]. Dementia is a progressive and untreatable disease. Lung fibrosis is the most damaging symptom contributing to the death of Covid-19 infection. Studies of wound healing metabolites on unhealed wounds may be helpful to save such fatal diseases.

Conclusions

Health professionals have the obligation to solve health problems contributing to death. Covid-19 is now the major concern. The solution of Covid-19 should be the first priority. Cancer was recognized by President Nixon as the main concern of health to declare "War on Cancer" in 1971. At that time, cytotoxic agents were the choice of cancer establishments to solve the most outstanding feature of cancer which was the perpetual cell replication. Cytotoxic agents, however, failed to win the war on cancer within the five years of a presidential project. Cancer establishments realized that cytotoxic agents could not win the war on cancer, thus, shifted the emphasis of cancer research from cytotoxic agents to DNA research, and gene and targeted therapies. Many excellent targeted agents were discovered which, however, could not compete with cytotoxic agents to cause the tumor to disappear, so the search turned to anti-angiogenesis, which also failed to produce cancer drugs to replace cytotoxic agents. The current emphasis was on immunotherapy, which has produced promising drugs for lung cancer. Immunotherapy, however, appears to have the same problems as the cytotoxic agents to cause the damage to chemo-surveillance and to show ineffectiveness against CSCs. These deleterious effects can be remedied by the employment of CDA formulations.

Wound healing metabolites are the nature's creation to ensure perfection of wound healing to avoid the evolution of PSCs to become CSCs. Cancer arises due to the collapse of chemo-surveillance, thus, wound healing metabolites are the most appropriate medicines to heal cancer. Wound healing metabolites may also be the most appropriate medicines for the therapy of untreatable diseases arising due to the collapse of chemo-surveillance such as dementia and tissue fibrosis.

Notes

Both authors agree to the content of this article.

Data reported were produced by Ming C. Liao or by others under his management, which have been published as cited.

Role of the funder: It is not applicable.

Author disclosure: None exist.

Author contribution: Ming C. Liao designed and performed the studies. He wrote the first draft. Christine L. Craig was Ming C. Liao's research assistant when he was the chairman of the Department of Biochemistry during 1980 to 1993 at Burzynski Research Institute of Stafford, Texas. She transferred figures from published articles to this article and edited the first draft to become the final version.

Disclaimers: There is no conflict of interest.

Acknowledgements: Studies of chemo-surveillance, and action mechanisms of DIs and DHIs were supported by Dr. Stanislaw R. Burzynski of Burzynski Research Institute of Stafford, Texas. Clinical development of CDA-2 was supported by Mr. Ringo M. L. Chang of Everlife Pharmaceutical Company of Hefei, Anfei, China. Identification of the tumor factor of abnormal methylation enzymes as the catalytic subunit of telomerase was supported by Professor George C. Y. Chiou of Texas A & M University Medical Center. Studies of CDA formulations were supported by Professor John P. Fruehauf of Chao's Family Comprehensive Cancer Center, University of California Irvine Medical Center.

References

1. Liao MC, Kim JH, Fruehauf JP. Potentiation of ATRA activity in HL-60 cells by targeting of methylation enzymes. *J PharmacolPharmaceuPharmacovigi*. 2019; 3:009. DOI: 10.24966/PPP-5649/100009.
2. Liao MC, Kim JH, Fruehauf JP. Destabilization of abnormal methylation enzymes. Nature's way to eradicate cancer stem cells. *Online J Complement Alt Med*. 2019; 2(5): 1-6. DOI:10.33552/OJCAM.2019.02.000546.
3. Liao MC, Baker LL. Cancer arises as a consequence of wound not healing properly. Thus, perfection of wound healing must be the most appropriate strategy to win the war on Cancer. *AdvComplement Alt Med*. 2021; 6(3); 584-586.
4. Virchow R. Die Cellular Pathologie in Ihrer Begrundung auf Physiologische und Pathologische Gewebelehve. *Hirschwald*.1858; 16, 440. German.
5. Dvorak HF. Tumors: Wounds that do not heal. *N Engl J Med*. 1986; 315(26): 1650-1659.
6. Liao MC, Lin GW, Hurlbert RB. Partial purification and characterization of tumor and liver S-adenosylmethionine synthetases. *Cancer Res*. 1977; 37(2): 427-435.
7. Liao MC, Chang CF, Giovanella BC. Demonstration of an altered S-adenosylmethionine synthetase in human malignant tumors xenograted into athymic nude mice. *J Natl Cancer Inst*. 1980; 64(5): 1071-1075.
8. Liao MC, Zhuang P, ChiouGCY. Identification of the tumor factor of abnormal methylation enzymes as the catalytic subunit of telomerase. *Clin Oncol Cancer Res*. 2010; 7(2):86-96.
9. Liao MC, Szopa M, Burzynski B, Burzynski SR. Chemo-surveillance: A novel concept of the natural defense mechanism against cancer. *Drug Exptl Clin Res*.1989; 13 (Suppl. 1) : 72-

82.

10. Liao MC, Baker LL. The functionality of chemo-surveillance dictates the success of wound healing as well as cancer therapy. *Novel Res Sci*. 2021; 7(2): 1-3.
11. Liao MC, Craig CL. Chemo-surveillance as a natural mechanism to ensure perfection of wound healing to avoid cancer evolution and to cure cancer. In: *New Horizons in Medicine and Medical Research*. 2022; Vol 6, Chapter 3. Print ISBN: 978-93-5547-607-4.
12. Liao MC, Lee SS, Burzynski SR. Hypomethylation of nucleic acids: A key to the induction of terminal differentiation. *Intl J Exptl Clin Chemother*. 1989; 2(1): 187-199.
13. Liao MC, Craig CL. On the mechanism of wound healing and the impact of wound on cancer evolution and cancer therapy. *Intl Res J Oncol*. 2021; 5(3): 25-31.
14. Liao MC, Baker LL. Wound healing, evolution of cancer and war on cancer. *Intl Res J Oncol*. 2021; 4(3): 13-20.
15. Liao MC, Craig CL. No scar as an indication of perfect wound healing, ugly scar as imperfect wound healing and cancer as failed wound healing. *J Cancer Tumor Intl*. 2022; 12(1): 29-34.
16. Liao MC, Baker LL. Destruction promotes the proliferation of progenitor stem cells and cancer stem cells. Therefore, non-destruction strategy is a better choice for cancer therapy. *J Pharmacol Pharmaceu Pharmacovigi*. 2020; 4:029. DOI: 10.24966/PPP-5649/100029
17. Liao MC, Baker LL. Cell differentiation agent formulations to win the war on cancer. *Cancer Sci Res*. 2022; 5(2): 1-4.
18. Ho ATV, Palla AR, Blake MR, YuceIND, Wang YX, Magnusson KEG, et al. Prostaglandin E2 is essential for efficacious skeletal muscle stem function, augmenting regeneration and strength. *Proc Natl Acad Sci USA*. 2017; 114(26): 6675-6684.
19. Itkin T, Rafii S. Leukemia cells "gas up" leaky bone marrow blood vessels. *Cancer Cell*. 2017; 32(3): 276-278.
20. Passaro D, Di Tullio A, Abarategi A, Rousault-Pierre K, Foster K, Ariza-McNaughton L, et al. Increased vascular permeability in the bone marrow microenvironment contributes to disease progression and drug response in acute myeloid leukemia. *Cancer Cell*. 2017; 32(3): 324-341.
21. Liao MC, Szopa M, Burzynski B, Burzynski SR. Quantitative assay of plasma and urinary peptides as an aid for the evaluation of cancer patients undergoing antineoplastic therapy. *Drugs Exptl Clin Res*. 1987; 13 (Suppl. 1) : 61-70.
22. Kamparath BN, Liao MC, Burzynski B, Burzynski SR. Protective effect of antineoplastic A10 in hepatocarcinogenesis induced by aflatoxin B₁. *Intl J Tiss React*. 1990; 12 (Suppl): 43-50.
23. Williamson PJ, Kruger AR, Reynolds PJ, Hamlin TJ, Oscier DG. Establishing the incidence of myelodysplastic syndromes. *Br J Haemato*. 1994; 87(4): 743-745.
24. Boula A, Vougarelis M, Giannouli S, Katrinakis G, Psyllaki M, Pontikoglou C, et al. Effect of CA2 of antitumor necrosis factor-alpha antibody therapy on hematopoiesis of patients with myelodysplastic syndromes. *Clin Cancer Res*. 2006; 12(10): 3099-3108.
25. WolflPS, Kjallquist U, Chowdhury O, Doolittle H, Wedge DC, Thongjuea S, et al. Myelodysplastic syndromes are propagated by rare and distinct human cancer stem cells in vivo. *Cancer Cell*. 2014; 25(6): 794-808.
26. Liao MC, Chang CF, Becker FF. Alteration of S-adenosylmethionine synthetases during

- chemical hepatocarcinogenesis and in resulting carcinomas. *Cancer Res.* 1979; 39(6): 2113-2119.
27. Liao MC, Baker LL. Abnormal methylation enzymes as the bullseye of targeted cancer therapy. *Novel Res Sci.* 2021; 7(4): 1-3
 28. Bacanelli AC, Turner FB, Xie LY, Taylor SM, Moran RG. A mouse gene that coordinate epigenetic controls and transcriptional interference to achieve tissue specific expression. *Mol Cell Biol.* 2008; 28(2): 836-848.
 29. Liao MC, Hunt ME, Hurlbert RB. Role of ribosomal RNA methylases in the regulation of ribosome production. *Biochemistry.* 1976; 15(14): 3158-3164.
 30. Bernstein KA, Bleichert F, Bean JM, Cross FR, Baserga SJ. Ribosome biogenesis is sensed at the start cell cycle check point. *Mol Biol Cell.* 2007; 18(3): 953-964.
 31. Justilien Y, Ali SA, Jamieson L, Yin N, Cox AD, Der CJ, et al. ECT2-dependent rRNA synthesis is required for KRAS-TRP53-driven lung adenocarcinoma. *Cancer Cell.* 2017; 31(2): 256-269.
 32. Liao MC, Chang CF, Saunderson GF, Tsai YH. S-adenosylhomocysteine hydrolases as the primary target enzymes in androgen regulation of methylation complexes. *Arch Biochem Biophys.* 1981; 208(1): 261-272.
 33. Liao MC. Abnormal methylation enzymes: A selective molecular target for differentiation therapy of cancer. *Chin Pharm J.* 2004; 56(2): 57-67.
 34. Prudova A, Bauman Z, Braun A, Vitvitsky V, Lu SC, Banerjee R. S-Adenosylmethionine stabilizes cystathionine beta-synthase and modulates redox capacity. *Proc Natl Acad Sci USA.* 2006; 103(17): 689-6494.
 35. Chiba P, Wallner C, Kaizer E. S-Adenosylmethionine metabolism in HL-60 cells: Effect of cell cycle and differentiation. *Biochim Biophys Acta.* 1988; 971(1): 38-45.
 36. Liao MC, Kim JH, Fruehauf JP. Destabilization of abnormal methylation enzymes to combat cancer: The nature's choice to win the war on cancer. *Lambert Academic Publishing.* 2020 International Book Market Service Ltd, 978-620-2-66889-7.
 37. Liao MC. Pharmaceutical composition inducing cancer cell differentiation and the use for treatment and prevention of cancer thereof. *US Patent 7232578.B2,* 2007.
 38. Liao MC, Fruehauf PA, Zheng ZH, Fruehauf JP. Development of synthetic cell differentiation agent formulations for the prevention and therapy of cancer via targeting of cancer stem cells. *Cancer Stu Ther J.* 2019, 4(1): 1-15.
 39. Feng F, Li Q, Ling CQ, Zhang Y, Qin F, Wang H, et al. Phase III clinical trials of the cell differentiation agent-2(CDA-2): Therapeutic efficacy on breast cancer, non-small cell lung cancer and primary hepatoma. *Chin J Clin Oncol.* 2005; 2(4): 706-716.
 40. Prassana P, Shack S, Wilson VL, Samid D. Phenylacetate in chemoprevention of 5-aza-2'-deoxycytidine-induced carcinogenesis. *Clin Cancer Res.* 1995; 1(18): 865-871.
 40. Gaudet F, Hodgeson JG, Eden A, Jackson-Grusby L, Dausman J, Gray JW, et al. Induction of tumor in mice by genomic hypomethylation. *Science.* 2003; 300(5618): 489-492.
 41. Pali SS, van Emburgh BO, Sankpal UP, Brown KD, Robertson KD. DNA methylation inhibitor 5-aza-2'-deoxycytidine induces reversible DNA damage that is distinctly influenced by DNA-methyl-transferase 1 and 3B. *Mol Cell Biol.* 2008; 28(2): 752-771.
 43. Kizietepe T, Hideshima T, Catley L, Raje N, Yasui H, Shiraishi N, et al. 5-Azacytidine, a methyltransferase inhibitor, induces ATR-mediated DNA-double strand break responses,

- apoptosis, and synergistic cytotoxicity with doxorubicine and bortezomib against multiple myeloma cells. *Mol Cancer Ther.* 2007; 6(6): 1718-1727.
44. Yang Q, Wu F, Wang F, Cai K, Zhang Y, Sun Q, et al. Impact of DNA methyltransferase inhibitor azacytidine on cardiac development of zebrafish in vivo and cardiomyocyte proliferation, apoptosis, and the homeostasis of gene expression in vitro. *J Cell Biochem.* 2019; 120(10):17459-17471.
45. Liao MC, Huang Li, Lee JH, Chen SC, Kuo SC. Development of differentiation helper inducers for the differentiation therapy of cancer. *Chin Pharma J.* 1988; 50(5): 289-303.
46. Liao MC, Kim JH, Fruehauf JP. In pursuance of differentiation inducers to combat cancer via targeting of abnormal methylation enzymes. *J Cancer Tumor Intl.* 2020; 10(2): 39-47.
47. Liao MC, Kim JH, Fruehauf JP. Arachidonic acid and its metabolites as the surveillance differentiation inducers to protect healthy people from becoming cancer patients. *Clin Pharmacol Toxicol Res.* 2021; 4(1): 7-10.
48. Liao MC, Fruehauf JP. The winner of the contest to eradicate cancer stem cells wins the the contest of cancer therapies: The winner is cell differentiation agent formulations. *Adv Complement Alt Med.* 2020; 5(4): 476-478.
49. Neurology Editorial Committee, European Society of Neurology. Trends and Innovations in Dementia. *Personal communication.* May 23, 2022.
50. Wynn TA. Cellular and molecular mechanisms of fibrosis. *J Pathol.* 2008; 214(2): 199-210.
51. Rybinski B, Cukieman E. The wound healing, chronic fibrosis, and cancer progression triad. *Physiol Genomics.* 2014; 46(7): 223-244.

