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Review

Mechanisms of cryoablation: Clinical consequences on malignant tumors

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ABSTRACT

While the destructive actions of a cryoablative freeze cycle are long recognized, more recent evidence has revealed a complex set of molecular responses that provides a path for optimization. The importance of optimization relates to the observation that the cryosurgical treatment of tumors yields success only equivalent to alternative therapies. This is also true of all existing therapies of cancer, which while applied with curative intent; provide only disease suppression for periods ranging from months to years. Recent research has led to an important new understanding of the nature of cancer, which has implications for primary therapies, including cryosurgical treatment. We now recognize that a cancer is a highly organized tissue dependent on other supporting cells for its establishment, growth and invasion. Further, cancer stem cells are now recognized as an origin of disease and prove resistant to many treatment modalities. Growth is dependent on endothelial cells essential to blood vessel formation, fibroblasts production of growth factors, and protective functions of cells of the immune system. This review discusses the biology of cancer, which has profound implications for the diverse therapies of the disease, including cryosurgery. We also describe the cryosurgical treatment of diverse cancers, citing results, types of adjunctive therapy intended to improve clinical outcomes, and comment briefly on other energy-based ablative therapies. With an expanded view of tumor complexity we identify those elements key to effective cryoablation and strategies designed to optimize cancer cell mortality with a consideration of the now recognized hallmarks of cancer.

Introduction

Cryoablative therapies rely on controlled, local freezing caused by the removal of thermal energy (heat) from the tissues; hence, an energy-deprivation strategy. These procedures are grounded on well-established cryobiological principles linked to the investigative work on the cryopreservation of cells and pathogenesis of frostbite. Cooper [29,30] first described a necrotic outcome, provided a tissue temperature of −20 °C or colder, held for 1 min or longer, was attained. This description in terms of nadir temperature and freeze duration provided the basis for cryoablative dosimetry and was a key to early clinical successes. Numerous other experimental and clinical reports have attempted to define the cryoablative dose in terms of temperature–time to assure complete tissue destruction. However, a precise definition of the "cryoablative dose" is difficult due to the diversity of opinions and practices as they related to procedural implementation, the existence of thermal gradients in frozen tissue (often related to cryoprobe performance characteristics), variations in regional blood flow, anatomical distinctions, the use of accessory warming device, cancers distinct phenotypic responses to a freeze–thaw stress, and the molecular signaling (survival and cascades death) of cells.

From a conceptual perspective, the idea of dosing originally relied on the presumption that only physical parameters related to the freeze–thaw cycle and tumor's capillary support structure were determinate of cancer cell survival. In 1999 the first reports appeared implicating post-thaw cell stress responses as equally important to managing tumor ablation through gene regulated cell death pathways [60]. An evaluation of a variety of studies allows one to conclude that inhibition of survival stress signaling pathways with adjunctive agents can enhance the ablative effect of freezing. Further, studies have also shown that optimization of the physical factors associated with cryoprocedures can similarly

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affect treatment-dependent cell death [11]. Since a cancer cell population response to temperature excursions is typically "normal" or Gaussian [121], there is a population of cancer cells that may avoid freeze rupture and evoke cell survival mechanisms (pathways) to avoid apoptosis and secondary necrosis. This cancer cell survival response requires disruption for assured treatment efficacy. In practice, there is one caveat to the concept of predictable dosing. Accurate tumor temperature measurement can be difficult due to thermocouple placement variation and even error and the impact of thermocouple positioning adjacent to blood vessels (heat sources). Hence, the temperature thresholds provided in this review are those "commonly accepted" within the field.

Numerous ablative therapies are currently in use for the treatment of cancers. While applied with curative intent, these therapies provide only modest success in disease suppression, not cure, as cancer cell mutation often enables resistance to therapy. This outcome is despite a half century of research revealing only modest improvements in durable response to diverse treatment strategies [32]. This disappointing absence of cure is related to the fact that "cancer" represents a group of more than 150 diseases linked to cellular genetic controls exhibiting defensive strategies with clear mutagenic responses.

A tumor is no longer considered a homogeneous mass of cells, growing without replicative control, causing the disruption of the architecture of primary and numerous secondary (metastatic) sites. Tumors are highly organized structures dependent on supporting cells for their establishment, growth and invasion. A hierarchy of intercellular commands provides for an "orderly" progression of the disease with accompanying defensive strategies that compromise both natural immunity and additive therapeutic interventions (i.e. radiation, chemotherapy, etc.) [31]. Cancer stem cells, now recognized as a potential origin of a tumor, lend unanticipated resiliency to the disease.

Three sentinel changes in our understanding of cancer are *in process*. First, cancer stem cells (CSC) [20] are now accepted as key elements of tumorigenesis as well as a "cell-of-origin" (a mutated tissue stem cell) and can be highly resistant to radiation [49] and chemotherapy [128]. Second, tumor formation involves the recruitment of numerous non-cancer support cells that establish a microenvironment essential to tumor survival, growth and ultimate metastasis. These tumor-associated cells include endothelial cells essential to blood vessel formation, fibroblasts to serve various support functions. Third, cells of the immune system assume a protective role for the cancer cells by masking cancer immunogenicity from circulating immune cells (i.e. macrophages, etc.), nutritive serosal cells and mesenchymal cells [54]. Hence, the tumor microenvironment creates a protective neo-tissue environment that can serve to isolate the tumor from the various defense strategies of the body. Linked to each of the above is the growing body of evidence demonstrating that with successive therapeutic attempts, the cancer cells acquire progressively enhanced resistance to individual therapeutic modalities (i.e. chemotherapy, radiation, hormonal deprivation, etc.). For example, exposure to successive bouts of cytotoxic drugs results in the survival of approximately 20–30% of the population of the cancer cells as only those cells in dividing stages succumb to the toxic exposure. With follow up treatments each additional dose result in tumor-associated fibroblasts secreting a surface protective protein (Wnt 16B) which enhances cancer cell chemotherapeutic resistance [128]. In addition, other defensive strategies are brought into play such as the upregulation of membrane protein pumps that function to eliminate the chemotherapeutic agent. Radiation has also been shown to induce the same Wnt 16B response as well as yield the amplification of DNA repair/protective strategies and inhibition of apoptosis [49].

Cryoablation is unique as a treatment modality in that it is typically a monotherapy applied without follow up or successive treatments thereby denying cancer cells the opportunity to develop defensive mutations. Additionally, the freeze–thaw process results in a disruption of many of the principal characteristics, hallmarks, of cancer [55] (Table 1). These hallmarks are indicative of evolved capabilities of cancer to assure successful tumor growth in the face of diverse, well-established anti-tumor protective adaptations. Being an energy-deprivation therapy, cryoablation has allowed the opportunity to extend our understanding to include activation of freeze-induced molecular stress cascades and their manipulation. These strategies are providing a new therapeutic path that holds promise for improved patient outcomes.

This review will evaluate and project principals to alter and improve today's techniques for cryosurgery, recognize the inherent variability implicit in a cryoablative application and how that variability might prove beneficial, and provide insight into adjunctive strategies designed to increase the efficacy of cryosurgery.

Cryoablative injury mechanisms

Dosimetry

For nearly forty years since the 1960's work of Cooper, significant and logical attempts have been expended in an effort to describe a cryosurgical "ablative dose." The localized, sharply demarcated zone of the "ice ball" is *a priori* considered "lethal" especially when a second freeze–thaw cycle is included in the procedure (Fig. 1). Reports have described the thermal distribution of the edge of the iceball as viewed under ultrasound with the leading edge of the hyperechoic rim by ~0 °C and the trail edge ~−15 °C to −20 °C [5].

If this concept was accurate, the "ablative dose" would simply equal the volume of frozen tissue located concentric to a given isotherm. Cooper [29] defined the "lethal dose" as −20 °C for 1 min. Later Neel [93] re-defined the lethal dose as −60 °C where as Staren et al. [126] identified −70 °C as the target temperature (refer to Clinical Application section). These reports, however, did not adequately address the issue of time-at-temperature. More recently, −40 °C has emerged as the target temperature based on a variety of *in vitro* and animal studies as well as the physics of pure water which supports the suggestion that small volumes ("cell sized") of liquid water do not have the ability to undercool (remain a liquid) much below −40 °C. Accordingly, this suggests that all freezable liquid water in a cell would be expected to crystallize near −40 °C resulting in the formation of lethal intracellular ice. Taken together, our knowledge of the physics of water, our nascent understanding of the biology of cells at low temperatures and the conclusions drawn from pre-clinical experimentation, it is often taught that −40 °C for "a few minutes" represents a targeted "lethal dose" [42]. Several *in vitro* based studies using a variety of cancer cells have further supported the notion that targeting

Table 1
Hallmarks of cancer-classifications of generally recognized survival strategies used by cancer.

- Growth – sustained and proliferative
- Evasion of growth suppressors
- Avoidance of cell death pathways
- Reproductive immortality
- Induction of angiogenesis
- Activation of tissue invasion and metastasis
- Reprogrammed energy metabolism
- Evasion of immune destruction
- Establishment of tumor microenvironment

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