Original Paper

Pathobiology

Pathobiology 1999;67:148–157

Received: February 25, 1999
Accepted: March 8, 1999

Three-Dimensional Spheroid Model in Tumor Biology

Maria Teresa Santini Gabriella Rainaldi

Laboratorio di Ultrastrutture, Istituto Superiore di Sanità, Rome, and Istituto Nazionale per la Fisica della Materia, Unità di Napoli, Università di Napoli 'Federico II', Complesso Universitario Monte S. Angelo, Naples, Italy

Key Words
Multicellular spheroids · Tumors · Cell death

Abstract
It is becoming more and more apparent that monolayer cultures of tumor cells cannot completely represent the characteristics of three-dimensional solid tumors. Consequently, the multicellular tumor spheroid model, which is of intermediate complexity between in vivo tumors and monolayer cultures, was developed. In this review, the major similarities between spheroids and solid tumors are discussed. After a brief survey of the different spheroid culturing techniques, the general morphological and growth characteristics of these systems are examined and compared to solid tumors. Finally, selected studies regarding the use of tumor spheroids to examine cell response to antineoplastic agents and radiation, cell death including both necrosis and apoptosis and cell adhesion in spheroids are reviewed.

Introduction

For many years, cell biologists have investigated cancer, primarily by using stabilized tumor cell lines grown in monolayer. Although this in vitro cell model has yielded much valuable information regarding the mechanisms at the basis of malignant growth, it is, nonetheless, unsuitable in representing completely in vivo tumors. It should be recalled that solid tumors grow in a three-dimensional spatial array and that the cells in these tumors are exposed to non-uniform distributions of oxygen and nutrients as well as other physical and chemical stresses. Consequently, because of the great microenvironmental variations present in different regions of tumors, significant cellular heterogeneity may result. In fact, in zones where oxygen and nutrient supplies are low, cell damage and even necrosis may develop. From these considerations, it is quite apparent that bidimensional growth of cancer cells, in which all of the cells are equally exposed to oxygen and nutrients, cannot be used in examining all aspects of tumor biology. In addition, it may also be hypothesized that many of the differences in response to radio- and chemotherapy observed between cells grown in monolayer and those found in in vivo tumors may be the direct result of the differences in spatial organization and cell-cell contacts. Thus, in an attempt to design more suitable in vitro systems which take into consideration the three-dimensional arrangement of solid tumors, multicellular tumor spheroids have been developed. In fact, tumor spheroids represent quite realistically the three-dimensional growth and organization of solid tumors and, consequently, simulate much more precisely the cell-cell interactions and microenvironmental conditions found in these tumors. In

KARGER
Fax + 41 61 306 12 34
E-Mail karger@karger.ch
www.karger.com

1015–2008/99/0673–0148$17.50/0

Accessible online at:
http://BioMedNet.com/karger

Dr. Maria Teresa Santini
Laboratorio di Ultrastrutture, Istituto Superiore di Sanità
Viale Regina Elena, 299, I–00161 Rome (Italy)
Tel. +3906 4990 2817, Fax +3906 4938 7140
E-Mail santini@iss.it

addition, they can be grown using many different cell types, are easy to handle in the laboratory and offer a very high level of reproducibility.

Historically, the use of spherical cell aggregates or spheroids as an in vitro culture model began many years ago. Aggregates of embryonic cells were developed first and only later were tumor cells utilized. In fact, the pioneering work conducted by Holtfreter was concerned primarily with using embryonic cell aggregates to examine amphibian development [1, 2] while Moscona studied both embryonic [3] and later cancer cell aggregates [4]. In a subsequent work, Moscona [5] proposed a technique for the formation of multicellular spheroids which consisted in placing single-cell suspensions in Erlenmeyer flasks on gyratory shakers. This technique was later utilized by other workers to form tumor spheroids in cancer research [6, 7] as well as myoblast aggregates and spheroids to study muscle development [8–10]. Investigations with multicellular tumor spheroids continued with Sutherland and coworkers who made many important observations with these systems not only regarding radiation biology [11, 12], but also concerning fundamental research in cell biology and cancer [13, 14].

In this review, the major similarities between multicellular tumor spheroids and in vivo tumors will be discussed. In particular, after a brief survey of the different spheroid culturing techniques, the general characteristics of these three-dimensional model systems, including morphology and growth, will be examined and compared to solid tumors. After this general overview, selected studies representing what the authors feel to be some of the major areas of application of spheroids in cancer research will be reviewed. Specifically, the use of tumor spheroids to study cell response to antineoplastic agents and radiation, cell death including both necrosis and programmed cell death (apoptosis) and cell adhesion in spheroids and cancer will be discussed. It is hoped that this brief review will stimulate the use of the multicellular tumor spheroid model in investigating various aspects of tumor biology as well as of more basic cellular mechanisms.

Spheroid Culturing Techniques

Liquid-Overlay Technique

Various methods may be used to obtain in vitro cultures of spheroids. The liquid-overlay technique is the most widely utilized. It involves placing trypsinized, monolayer-cultured single tumor cells or single-cell suspensions of trypsinized tumor tissue in dishes covered with a non-adhesive surface. A thin layer of agar or agarose is often used, although an agarose film is preferred by many investigators [15]. Alternatively, bacterial grade plastic culture dishes, in which cell adhesion does not occur, may also be used. The single cells placed on these surfaces do not adhere to the substratum, but continue to grow on top of it. In many cases, small colonies of aggregated cells form and for most human cell lines, aggregates form within 1–3 days. When the aggregates become well rounded, compacted and regular in shape, they are placed in clean agarose-covered dishes and kept in culture, with the appropriate medium changes, until they reach the desired dimensions. Single cells which have not formed aggregates and irregularly shaped aggregates are discarded. Figure 1 shows a phase contrast micrograph of spheroids formed in our laboratory from human mammary adenocarcinoma MCF-7 cells using the liquid-overlay technique with agar. Single cells were grown on agar for 3 days and then transferred to clean Petri dishes also covered with agar. Spheroids kept on the clean dishes for an additional 4 days are shown. As can be seen, the spheroids appear well rounded and quite regular in shape. The main disadvantage of using this technique is that the cells come into direct contact with agar or agarose when these are used, and that, consequently, changes in the surface properties of the cells may occur. The possible effects of these surface variations on spheroid cell structure and function should not be ignored.

Fig. 1. Phase contrast micrograph of human mammary adenocarcinoma MCF-7 spheroids grown for 7 days using the liquid-overlay technique using agar. Cells were grown on agar for 3 days and then transferred to clean Petri dishes also covered with agar and kept for an additional 4 days. × 250.

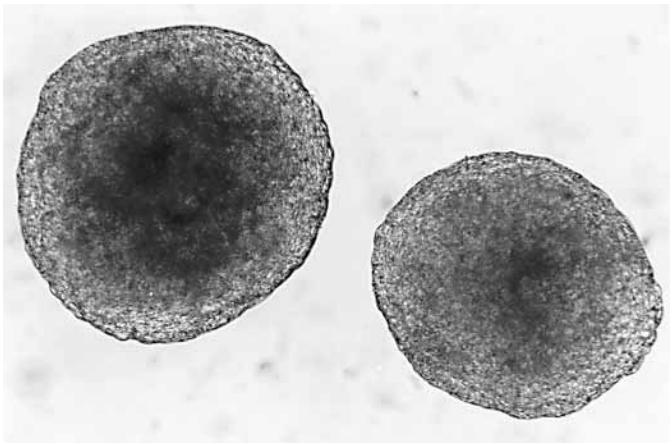

Fig. 2. Phase contrast micrograph of human epidermal squamous carcinoma A431 spheroids grown for 7 days using the gyratory rotation method. × 500.

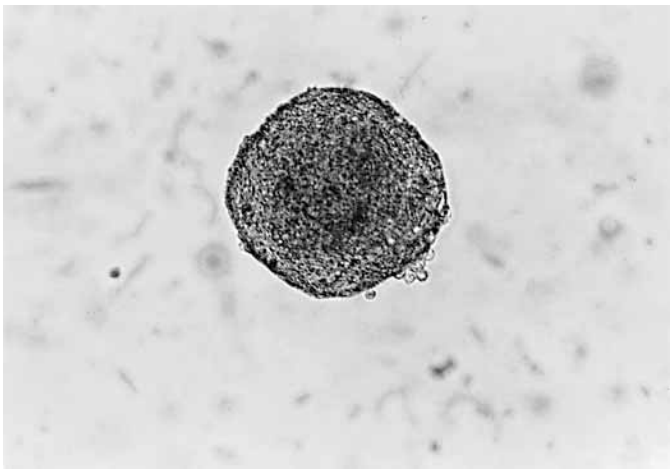

Fig. 3. Phase contrast micrograph of a human colon adenocarcinoma HT29 spheroid grown for 4 days on agar and an additional 7 days in rotation culture. × 250.

Spinner Flask Method

With the spinner flask method, spheroids are initiated with monolayer culture cells in the exponential growth phase [16]. When the cells are in this phase, they are collected by trypsinization. They are then placed in spinner flasks containing the proper growth medium which has been equilibrated with an air/CO_2 gas mixture. A magnetic stirrer is also placed in the flask and made to rotate at about 190 rpm. This stirring inhibits adhesion of the cells to the flask and maintains the cells in suspension. Homotypic aggregation and subsequent formation of the spheroids takes place with time in these suspended cells. The growth medium is changed when necessary and replaced with fresh medium. Often, only part of the medium is removed so that the concentrations of the various growth factors released by the cells into the medium itself are not altered dramatically. The spheroids are collected when they reach the desired size.

Gyratory Rotation System

This method, which was first utilized by Moscona [5] with embryonic cells, has also proven to be quite successful in the formation of cancer spheroids. It consists in placing a trypsinized suspension of monolayer tumor cells in glass or non-adhesive bacterial grade plastic Erlenmeyer flasks containing a carefully calculated amount of the proper growth medium [9]. It is necessary to determine this amount based on the total cell number quite precisely, otherwise aggregates and then spheroids will not form. Since the flasks are gas impermeable, before sealing them for sterility, an air/CO_2 gas mixture is added. The flasks are then placed in a temperature-controlled gyratory rotation incubator. The rotation rate based on the size of the culture flasks utilized must also be carefully determined. In most cases, spheroids form within a day. The spheroids are collected when they have reached the dimensions required. Figure 2 depicts a phase contrast micrograph of human epidermal squamous carcinoma A431 spheroids grown by the gyratory rotation method for 7 days. Spheroids appear well rounded and regular in shape.

It should also be pointed out that combinations of these techniques may also be used to obtain spheroids. For instance, we have utilized a combination of the liquid-overlay with agar and gyratory rotation methods to obtain spheroids of human colon adenocarcinoma HT29 cells. A spheroid grown for 4 days on agar and an additional 7 days in rotation is shown in the phase contrast micrograph of figure 3. As in the previous two figures, the spheroid formed from these cells also appears well rounded and regular in shape. However, due caution should be exercised when adopting these combinations.

As is apparent from the above paragraphs, spheroids from different cell lines may be formed by various techniques. However, the gyratory rotation method is the method preferred in our laboratory, particularly for studies of the surface of the cell membrane [17]. We feel that it is important that the cells do not come into direct contact with any foreign substances. In addition, this technique allows the formation of a great number of spheroids

grown under the same exact conditions simultaneously. If spheroids of the same size are needed, these may be chosen from the large spheroid population available. We also believe that the gyratory rotation method is less time-consuming. For example, HT29 spheroids can be grown by this technique much more rapidly and without manipulation than other techniques. In fact, the same spheroid size (about 150 μm) can be reached in 6 days directly with gyratory rotation while 8–9 days and 11 days are necessary with the liquid-overlay and combination methods, respectively. Finally, the information obtained with spheroids formed with the rotation technique is more reproducible over longer periods of time than other methods.

General Characteristics of Spheroids

Morphology

Three-dimensional tumor spheroids can be formed from an almost unlimited number of cell lines or tissues depending on the study to be conducted. The time course of spheroid formation from cell line to cell line, however, may vary. The temporal sequence of events leading to spheroid formation in human colon carcinoma HT29 cells from our laboratory is shown in figure 4. As can be seen in the phase contrast micrographs of figure 4, during the first hours of formation (up to 24 h; fig. 4a), there are no spheroids present, but only loose cell aggregates. However, with time (48 h), spheroids begin to take shape, but the cells are still loosely organized (fig. 4b). With still more time (5 days), spheroids form completely and become well-rounded structures composed of numerous, highly compacted cells in which it is difficult to distinguish individual cells from each other (fig. 4c). It is intuitive that this type of three-dimensional, compacted organization of the cells, which resembles that found in solid tumors, may have numerous consequences on the cells themselves, especially with respect to cell-cell contact and cellular communication. These cellular interrelationships may not be so well represented by monolayer cultures.

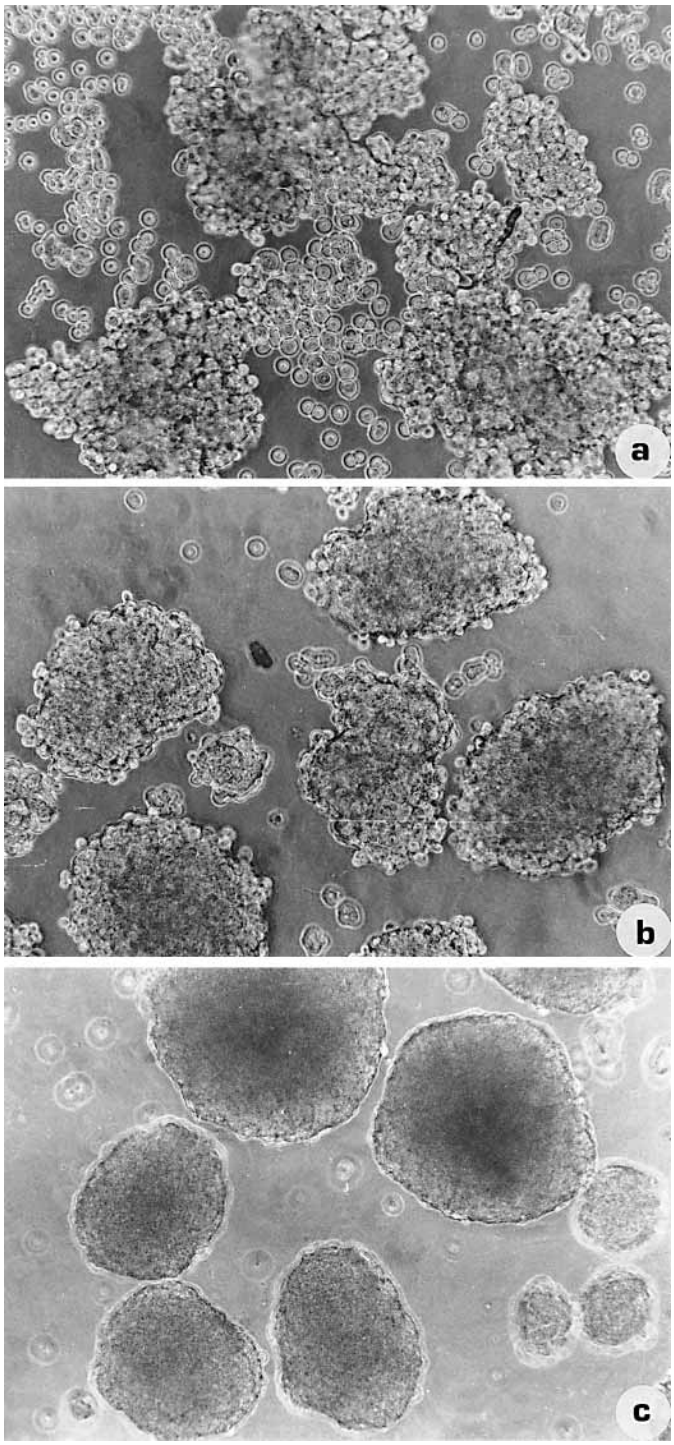

Fig. 4. Phase contrast micrographs illustrating the temporal dynamics of HT29 spheroid formation using the gyratory rotation technique. After 24 h of culture (**a**), loose cell aggregates are present in which individual cells can be distinguished from each other. After 48 h (**b**), spheroids begin to take shape, but the cells are still loosely organized. In the center of the aggregates, it is difficult to recognize individual cells while in the periphery single cells can still be easily distinguished. After 5 days (**c**), spheroids are completely formed and become well rounded with highly compacted cells. × 250.

Fig. 5. Scanning electron micrograph of A431 spheroids grown for 6 days by the gyratory rotation method. Spheroids appear regular in shape, have a smooth surface and do not have any filamentous substance covering this surface. × 170.

In most cases, the outer surface of well-formed tumor spheroids appears quite smooth. In fact, as can be seen in the scanning electron micrograph of A431 spheroids after 6 days of growth (fig. 5), the surface is quite smooth and it is difficult to distinguish individual cells from each other. However, in some cell lines (e.g. HT29 cells), although the surface is smooth, it can be covered by a thick filament network intimately associated with the surface itself (fig. 6a). This is especially evident at higher magnification (fig. 6b). However, even in HT29 cells, in areas where filaments are not present, the spheroid surface appears quite smooth (fig. 6c, asterisks) and it is difficult to distinguish individual cells. It is interesting to note the high similarity between three-dimensional tumor cell spheroids and in vivo solid tumors from the same tissues of origin. In fact, in in vivo squamous carcinomas, filamentous substances cannot be observed, as was the case in A431 spheroids. However, when in vivo colon carcinomas are studied with light microscopy, a filamentous substance similar to the one present in HT29 spheroids can be detected [18]. Further studies are underway in our laboratory in order to identify the nature of the substance released by HT29 cell spheroids and to ascertain whether it is constituted by extracellular matrix components, the production of which has been observed in close association with cell growth in spheroids by other authors [19]. It is also important to point out that no such substance was observed in HT29 cells grown in monolayer. Either it is not released by these cells or it produced in such low amounts that it cannot be

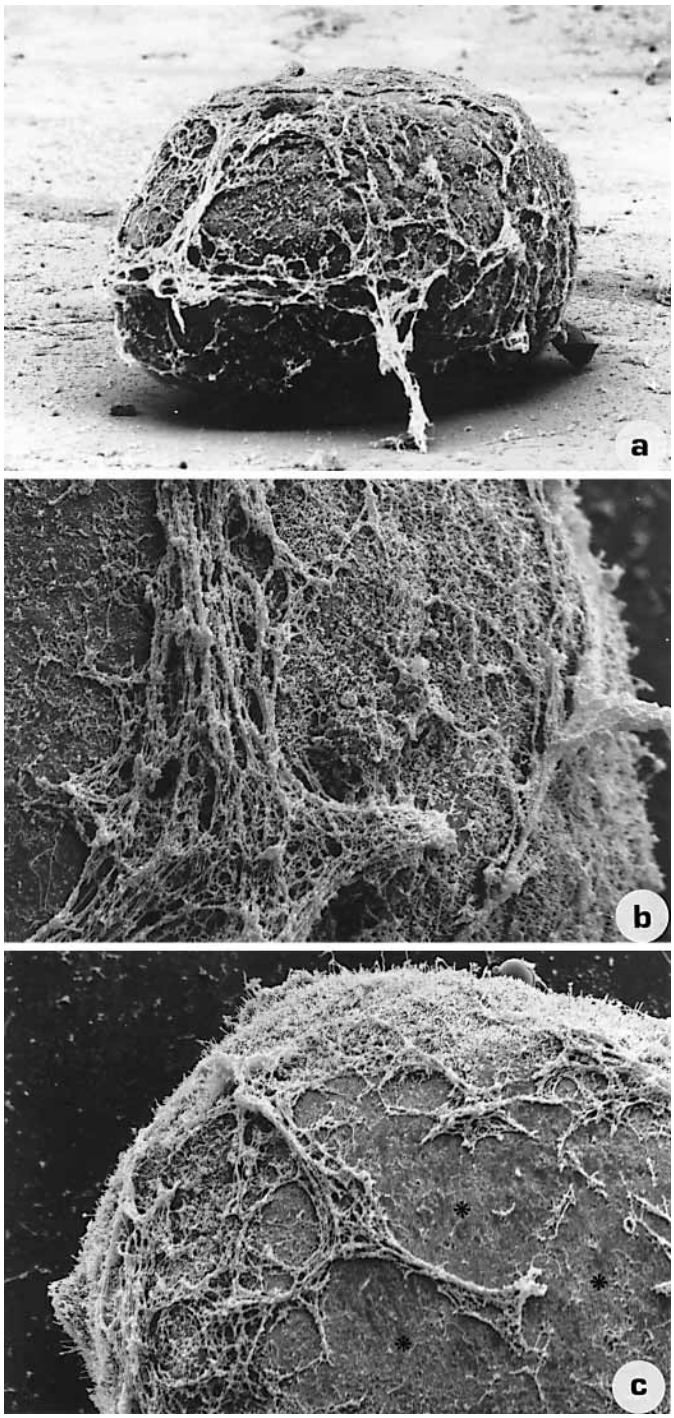

Fig. 6. Scanning electron micrographs of HT29 spheroids grown for 6 days using the gyratory rotation method. As can be seen in (**a**), the surface appears quite smooth although it is covered by a thick filament network. This is especially evident at higher magnification (**b**) even if in areas where filaments are not present, the spheroid surface appears quite smooth (**c**, asterisks). **a** × 350. **b** × 1,550. **c** × 850.

observed. An increased production of extracellular matrix components has been observed in glioma cells grown as spheroids with respect to monolayer cultures of the same cells [20]. Thus, it is apparent from the data presented that three-dimensional tumor spheroids resemble much more closely the morphology of in vivo tumors of the same tissue origin than monolayer cultures of the same cells.

Another aspect of multicellular tumor spheroid morphology which resembles in vivo tumors is their capacity to develop necrotic cells in areas far from nutrient and oxygen supplies. However, in spheroids, it is possible to control not only the size of these three-dimensional systems, but also the formation of the necrotic central area. This is extremely important since spheroids suited to a particular experiment can be easily developed. Figure 7 displays a semithin section of the interior of a A431 tumor spheroid in which the tight compaction and the close interrelationships between cells is clearly visible. However, no necrotic center is present and the cells appear well preserved throughout the spheroid. In particular, no signs of damage appear to be present in the nuclei of the cells. In fact, the chromatin is uniformly distributed in the nuclei and the nucleoli are well evident and very electron dense indicating pronounced cell activity. Occasionally, cells in the early phases of the apoptotic process (chromatin marginalization below the nuclear membrane) are present (arrow). It should be pointed out that the spheroids presented here were formed in 3 days of gyratory-rotation growth and ranged in size from 100 to 150 μm in diameter. This period of growth does not result in a necrotic center although such a center would result if A431 spheroids were maintained in culture for much longer periods of time. Growth studies conducted by us on A431 spheroids demonstrated that the number of cells in these systems almost tripled in 6 days of growth. Thus, it can be assumed that if such active cell proliferation continued, the spheroids would reach much greater dimensions. Such larger dimensions would result in a greater difficulty for oxygen and nutrients to reach the cells located in the center of the spheroid thus leading to the formation of a necrotic central area. Consequently, the culture time after spheroid formation should be critically evaluated before beginning a study, and spheroid size should be established to suit particular experimental needs.

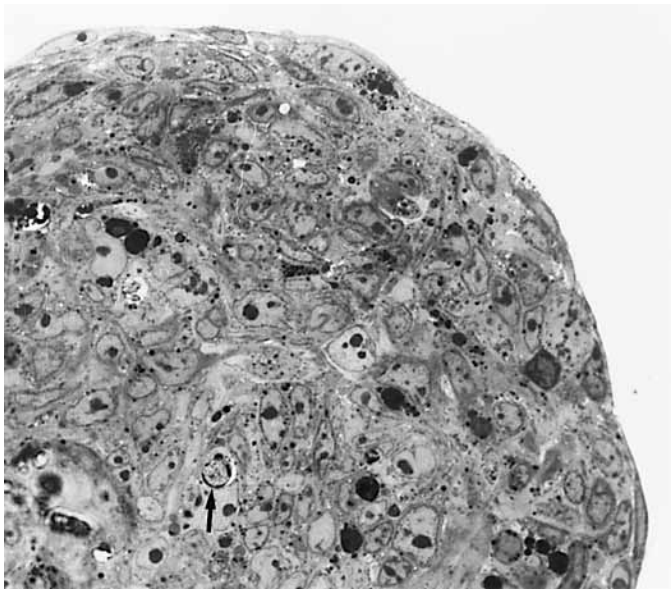

Fig. 7. Semithin section of a A431 spheroid grown for 3 days using the gyratory rotation method. Spheroids are tightly compacted and have no necrotic center. Occasionally, cells in the early phases of apoptosis (chromatin marginalization below the nuclear membrane) are present (arrow). × 2,000.

Growth

In addition to morphology, multicellular tumor spheroids also closely resemble in vivo solid tumors in their growth dynamics. Monolayer cultures grow exponentially while solid tumors are characterized by an early exponential phase followed by a period of retarded growth [21, 22]. The first phase is not dependent on external factors while the second depends on the size of the tumor and the nutritional restrictions imposed to cells located in the tumor interior. Multicellular tumor spheroids are also characterized by early exponential growth followed by a period of retarded growth [23]. In order to demonstrate the strong similarity that exists between solid tumor growth and growth of multicellular spheroids, various mathematical models have been applied. Growth curves with three successive phases (geometric, linear and plateau) have been described for spheroid growth [24]. The geometric phase corresponds to early aggregation and proliferation of small spheroids, while the linear and plateau phases represent the development of a nonproliferative inner region and the formation of a necrotic center in the spheroids, respectively. The exponential-Gompertzian tumor growth model has also been applied to several tumor cell lines grown in monolayer and as multicellular spheroids as well as to in vivo tumors [25, 26]. The results reveal that monolayer cultures do not adequately fit the model while both in vivo tumors and spheroids fit the model quite well and strongly resemble each other in their

growth characteristics. More recently, a system of nonlinear partial differential equations has been proposed to describe the avascular growth of tumor spheroids [27]. In this model, it is assumed that cells are present in two states (living and dead) both of which lead to volume changes in the spheroid and to the generation of a velocity field within the spheroid. The travelling-wave limit is formulated and analytical solutions are calculated. An exponential early phase followed by two growth retardation phases due to nutrient limitations and necrosis, respectively, are present. Computer modelling has also been used to describe cancer growth and applied to data from multicellular tumor spheroids [28]. Tumor growth in response to different radiotherapeutic protocols was also examined with this model. The results reveal that the model can be successfully applied to study the influences of treatment on the different cell populations present in tumors and spheroids in order to gain insight into the complex phenomenon of tumor growth. The main conclusion that may be drawn from the various models described is that multicellular tumor spheroids represent a much more realistic model for in vivo solid tumors than monolayer cultures and that these may be used successfully to study tumor growth in response to various radiotherapeutic treatments [29, 30].

Multicellular Spheroids as a Realistic Model to Study Tumor Response to Antineoplastic Agents and Radiation

Although the exact causes for the failure of chemotherapeutic and radiotherapeutic protocols are not yet clearly understood, similarities in the cellular mechanisms responsible for this failure appear to exist and may be studied more successfully with multicellular tumor spheroids. One of the major reasons that antineoplastic agents become ineffective in destroying tumors is that tumor cells become resistant to the drugs utilized. In some cases, this resistance also extends to other antineoplastic agents which have not been previously used (multidrug resistance). Multidrug resistance has been extensively studied with monolayer cultures of tumor cells and much valuable information has emerged [31, 32]. However, the data obtained with monolayer cultures is often in sharp contradiction to the observations made at the clinical level [33]. Consequently, in order to study both multidrug resistance as well as other types of drug resistance in an in vitro model which recreates tumor organization and growth more accurately, numerous studies with multicellular tumor spheroids have been conducted. For instance, it has been demonstrated that many tumor cell lines are much more resistant to antineoplastic agents if the cells are grown as three-dimensional spheroids rather than in monolayer culture [34, 35]. The resistance observed resembles much more closely the data obtained in the clinical setting. Although difficulty of drug penetration into the inner regions of the three-dimensional spheroid has been suggested as one of the principal causes for the increased resistance in spheroids and solid tumors [36], other factors also appear to exist. In fact, it has been shown that EMT-6 cells resistant to alkylating agents expressed their resistance if grown as multicellular tumor spheroids, but not if grown in monolayer [37]. From these and other data, it has been suggested that solid tumors may express acquired resistance at the multicellular level and not only at the cellular level [38].

Contradictions between clinical, radiotherapeutic results and those from radiation studies involving monolayer cultures have also been observed [39]. In radiation therapy, however, one of the causes for treatment failure is believed to be the existence of hypoxic cells which are resistant to radiation. These cells are usually located near the necrotic center of tumors where the microenvironment is much more acidic than more oxygenated zones. Thus, it is evident that the three-dimensional shape of multicellular tumor spheroids can provide a useful model for simulating the poorly oxygenated, necrotic areas of tumors and can be used to study the effects of radiation much more realistically than monolayer cultures. Santini et al. [40] have recently reviewed the use of multicellular spheroids in radiation biology.

It is postulated that the mechanisms which are responsible for resistance to radiation and acquired drug resistance in three-dimensional solid tumors may involve types of intercellular interactions which are not necessarily present in two-dimensional monolayer cultures [38]. In fact, the 'contact effect', together with hypoxia, has been been hypothesized to be one of the principal causes for the greater resistance of spheroids with respect to monolayer cells to ionizing radiation. This effect was first described by Durand and Sutherland [41] and has also been implicated in drug resistance [for a review, see ref. 42]. Among the various mechanisms hypothesized to be responsible for this effect is intercellular communication between cells [43], perhaps via gap junctions, which can permit recovery from both radiation as well as chemically induced cell damage. However, more recently, the involvement of gap junctions in the 'contact effect' in response to ionizing radiation has been excluded [44] and other mech-

anisms should be investigated. Modifications in cell shape, chromatin organization and intercellular signalling should be considered.

Cell Death in Multicellular Tumor Spheroids

Because of their many similarities with solid tumors, multicellular tumor spheroids also represent a very useful model for studying cell death, including both necrosis and programmed cell death (apoptosis). For instance, the development of necrotic areas in tumors has been extensively examined using spheroids and the concept that it is a restriction in the availability of oxygen and nutrients to determine these areas has been well documented by these studies. In fact, early investigations showed that reduction in the oxygen concentration in the medium surrounding spheroids and glucose availability as well as the accumulation of waste products play an important role in controlling the development of the central necrotic area [45–47]. More recent studies have confirmed these earlier results and have demonstrated that the depletion of substrates and accumulation of catabolites can explain formation of necrosis in spheroids without the assumption of any other additional mechanisms [48]. In addition, it has also been shown that energy metabolism is not involved in the formation of the necrotic core in spheroids since necrosis develops before the drop in energy-rich phosphate [49], and that the variable presence of hypoxia adjacent to necrotic areas in spheroids does not arise from stable genetic variants [50]. Among the more recent studies regarding glucose, the paper describing a new technique for measuring the glucose levels in distinct regions of spheroids is worth mentioning [51]. In fact, with this new method, it was possible to directly demonstrate the role of glucose in the development of cell heterogeneity and necrotic areas in spheroids as well as the higher capacity of tumor cells in spheroids for anaerobic rather than aerobic energy production. The role of hypoxia in regulating leukocyte activation and angiogenesis has also been investigated with multicellular spheroids [52]. An upregulation of interleukin 8 (IL-8), a cytokine with chemotactic and angiogenic properties, was observed in regions of low oxygen concentration in astrocytoma spheroids. A model where IL-8 production is induced first through inflammatory stimuli and later by a reduced oxygen concentration is suggested. An increase of IL-8 would promote angiogenesis and leukocyte infiltration and activation.

Besides the various aspects of necrotic death, apoptosis can also be studied in multicellular tumor spheroids. The study of programmed cell death is especially important not only for a more profound understanding of cell biology, but also if more efficacious antitumor protocols are to be designed. In fact, it is no longer enough to 'kill' tumor cells through highly destructive necrotic processes, but rather it would be much more useful to learn how to destroy them selectively through more 'natural' apoptotic pathways. For example, the possibility of improving the efficacy of radiotherapy by modulating apoptotic response was investigated by Dangles et al. [53] in monolayer and multicellular spheroids of three human bladder carcinoma cell lines. In particular, the role of epidermal growth factor (EGF) and transforming growth factor-α in affecting the apoptotic response of serum-starved or irradiated bladder cells grown in monolayer or as spheroids was examined. The paper demonstrates that EGF was able to inhibit apoptosis in spheroids by EGF receptor tyrosine kinase activation and that inhibitors of this receptor could be useful in sensitizing cancer cells and thus in improving the efficacy of radiotherapy. Apoptosis induced by antineoplastic agents can also be examined in three-dimensional spheroids. For instance, Frankel et al. [54] have shown that there is no difference in response between human ovarian cancer cell lines grown in monolayer or as spheroids to cisplatinum while spheroids are more resistant to Taxol than the same monolayer cells. In addition, monolayer cultures but not spheroids treated with Taxol showed an accumulation of cells in the G2-M phase as well as apoptotic death. From these results, it appears that the lack of apoptosis in spheroids after treatment with Taxol and the accompanying resistance of these three-dimensional model systems to this agent may be due to multicellular-dependent or associated mechanisms. Further studies are necessary in order to better comprehend the complex interrelationships between drug resistance, apoptosis and the cell cycle in tumor spheroids and in vivo solid tumors.

Although limited in number, the papers discussed above demonstrate that because of the many similarities to solid tumors, multicellular spheroids can be used quite successfully to examine both necrotic and apoptotic cell death. Much valuable information can be obtained by using this three-dimensional culture model.

Cell Adhesion and Spheroids

Cell-cell and cell-matrix interactions play a pivotal role in the development and spread of tumors. Although the precise mechanisms of the cellular interactions involved

in these processes are not yet clearly understood, the cell adhesion molecules (CAMs) appear to be directly involved. In particular, it has been demonstrated that one family of CAMs, the integrins, is extremely important in regulating numerous tumor cell functions such as cell-cell contact [55], invasion and metastatis [56] and anchorage-independent growth [57, 58]. Because of the many similarities which exist between solid tumors and multicellular spheroids, these in vitro model systems can be used to study the role of CAMs in tumors. In fact, Waleh et al. [59] have demonstrated that there is a downregulation of integrin receptors in spheroids of squamous cell carcinoma. In addition, other investigators [60] have shown that differences exist in the expression of integrin receptors in colorectal cells growing in monolayer, as multicellular spheroids or in nude mice. Together with the integrins, other surface molecules are also important in cancer [61–63] and, more recently, some of these have also been investigated in spheroids [17]. In this study, variations in the expression of CD44, I-CAM1 and LFA-3 were also observed in tumor cells grown in monolayer or as three-dimensional spheroids. The authors point out that because of the differences which exist in CAM expression as a consequence of the type of growth conditions, the results obtained should be critically evaluated before definite conclusions on the role of these molecules are made. This critical evaluation should be made especially when experiments are designed to examine the role of CAMs in cell function or in response to a particular treatment. Although the number of studies examining CAM expression in spheroids is limited, the results from these papers indicate that important information can be obtained with these model systems. Further studies on the role of CAMs in tumor biology are encouraged.

Conclusions and Future Perspectives

It is hoped that this review will stimulate interest in the use of spheroids in tumor biology and also in other areas of pathological research. Cell death and the role of adhesion in cancer are subjects which are especially revealing since in these types of studies the three-dimensional organization of spheroids is extremely important for the results obtained. Although research on these topics may be experimentally quite complex, every effort should be made to conduct such investigations so that more precise information regarding the effects of antineoplastic agents and radiation on tumor cell death and on the mechanisms responsible for tumor metastasis and invasion can be obtained.

Acknowledgements

The authors wish to thank Mr. Lamberto Camilli and Mr. Giacomo Monteleone for their excellent photographic work.

References

1 Holtfreter J: A study of the mechanics of gastrulation. J Exp Zool 1944;95:171–212.
2 Holtfreter J: Neural induction in explants which have passed through a sublethal cytolysis. J Exp Zool 1947;106:197–222.
3 Moscona A: Cell suspensions from organ rudiments of chick embryos. Exp Cell Res 1952;3:535–539.
4 Moscona A: The development in vitro of chimeric aggregates of dissociated embryonic chick and mouse cells. Proc Natl Acad Sci USA 1957;43:184–194.
5 Moscona A: Rotation-mediated histogenic aggregation of dissociated cells. Exp Cell Res 1961;22:455–475.
6 Halpern B, Pejsachowicz B, Febvre HL, Barski G: Differences in patterns of aggregation of malignant and non-malignant mammalian cells. Nature 1966;8:157–159.
7 Schleich A: Studies on aggregation of human ascites tumor cells. Eur J Cancer 1967;3:243–246.
8 Knudsen KA, Horwitz AF: Tandem events in myoblast fusion. Dev Biol 1977;58:328–338.
9 Santini MT, Indovina PL, Hausman RE: Changes in myoblast membrane order during differentiation as measured by EPR. Biochim Biophys Acta 1987;896:19–25.
10 Santini MT, Bonincontro A, Cametti C, Indovina PL: Cesium ions delay membrane fusion of chick embryo myoblasts in vitro: A conductivity study. Biochim Biophys Acta 1988;945:56–64.
11 Sutherland RM, Inch WR, McCredie JA, Kruuv J: A multicomponent radiation survival curve using an in vitro tumor model. Intl J Radiat Biol 1970;18:491–495.
12 Sutherland RM, Durand RE: Radiation effects on mammalian cells grown as an in vitro tumor model. Curr Top Radiat Res 1976;11:87–139.
13 Sutherland RM, MacDonald HR, Howell RL: Multicellular spheroids: A new model target for in vitro studies of immunity to solid tumor allografts. J Natl Cancer Inst 1977;58:1849–1853.
14 Sutherland RM, Carlsson J, Durand RE, Yuhas J: Spheroids in cancer research. Cancer Res 1981;41:2980–2994.
15 Carlsson J, Yuhas JM: Liquid-overlay culture of cellular spheroids; in Acker H, Carlsson J, Durand R, Sutherland RM (eds): Recent Results in Cancer Research. Spheroids in Cancer Research. Berlin, Springer, 1984, pp 1–23.
16 Sutherland RM, Durand RE: Growth and cellular characteristics of multicell spheroids; in Acker H, Carlsson J, Durand R, Sutherland RM (eds): Recent Results in Cancer Research. Spheroids in Cancer Research. Berlin, Springer, 1984, pp 24–49.
17 Rainaldi G, Calcabrini A, Arancia G, Santini MT: Different expression of adhesion molecules (CD44, ICAM-1 and LFA-3) in cancer cells grown in monolayer or as multicellular spheroids. Anticancer Res, in press.
18 Rosai J: Ackerman's Surgical Pathology, ed 8. St Louis, Mosby-Year Book, 1996.
19 Nederman T, Norling B, Glimelius B, Carlsson J, Brunk U: Demonstration of an extracellular matrix in multicellular tumor spheroids. Cancer Res 1984;44:3090–3097.

20 Glimelius B, Norling B, Nederman T, Carlsson J: Extracellular matrices in multicellular spheroids of human glioma origin: Increased incorporation of proteoglycans and fibronectin as compared to monolayer cultures. APMIS 1988; 96:433–444.
21 Laird AK: Dynamics of tumor growth. Br J Cancer 1964;13:490–502.
22 McCredie JA, Inch WR, Kruuv J: The rate of tumor growth in animals. Growth 1965;29: 331–347.
23 Sutherland RM: Cell and environment interactions in tumor microregions: The multicellular spheroid model. Science 1988;240:177–184.
24 Conger AD, Ziskin MC: Growth of mammalian multicellular tumor spheroids. Cancer Res 1983;43:556–560.
25 Demicheli R, Foroni R, Ingrosso A, Pratesi G, Soranzo C, Tortoreto M: An exponential-Gompertzian description of LoVo cell tumor growth from in vivo and in vitro data. Cancer Res 1989;49:6543–6546.
26 Demicheli R, Pratesi G, Foroni R: The exponential-Gompertzian tumor growth model: Data from six tumor cell lines in vitro and in vivo. Estimate of the transition point from exponential to Gompertzian growth and potential clinical implications. Tumori 1991;77: 189–195.
27 Ward JP, King JR: Mathematical modelling of avascular-tumour growth. IMA J Math Appl Med Biol 1997;14:39–69.
28 Düchting W, Ulmer W, Ginsberg T: Cancer: A challenge for control theory and computer modelling. Eur J Cancer 1996;32A:1283–1292.
29 Evans SM, Labs LM, Yuhas JM: Response of human neuroblastoma and melanoma multicellular tumor spheroids (MTS) to single dose irradiation. Int J Radiat Oncol Biol Phys 1986; 12:671–676.
30 Rofstad EK, Wahl A, Brusdat T: Radiation response of multicellular spheroids initiated from five human melanoma xenograft lines. Relationship to the radio-responsiveness in vivo. Br J Radiol 1986;59:1023–1029.
31 Wang Q, Beck WT: Transcriptional expression of multidrug resistance-associated protein (MRP) gene expression by wild-type p53. Cancer Res 1998;58:5762–5769.
32 Bradshaw DM, Arceci RJ: Clinical relevance of transmembrane drug efflux as a mechanism of multidrug resistance. J Clin Oncol 1998;16: 3674–3690.
33 Kaye SB: Multidrug resistance: Clinical relevance in solid tumors and strategies for circumvention. Curr Opin Oncol 1998;10:S15–S19.
34 Nederman T: Effects of vinblastine and 5-fluorouracil on human glioma and thyroid cancer cell monolayers and spheroids. Cancer Res 1984;44:254–258.
35 Olive PL, Banath JP, Evans HH: Cell killing and DNA damage by etoposide in Chinese hamster V79 monolayers and spheroids: Influence of growth kinetics, growth environment and DNA packaging. Br J Cancer 1993;67: 522–530.
36 Nederman T, Carlsson J, Malmquist M: Penetration of substances into tumor tissue – A methodological study on cellular spheroids. In Vitro 1981;17:290–298.
37 Kobayashi H, Man S, Kapitain SJ, Teicher BA, Kerbel RS: Acquired multicellular-mediated resistance to alkylating agents in cancer. Proc Natl Acad Sci USA 1993;90:3294–3298.
38 Kerbel RS, Rak J, Kobayashi H, Man MS, St. Croix B, Graham CH: Multicellular resistance: A new paradigm to explain aspects of acquired drug resistance of solid tumors. Cold Spring Harb Sympt Quant Biol 1994;59:661–672.
39 Kaaijk P, Troost D, Sminia P, Hulshof MC, van der Kracht AH, Leenstra S, Bosch DA: Hypofractionated radiation induces a decrease in cell proliferation but no morphological damage to organotypic multicellular spheroids of human glioblastomas. Eur J Cancer 1997;33: 645–651.
40 Santini MT, Rainaldi G, Indovina PL: Multicellular tumor spheroids in radiation biology. Int J Radiat Biol, in press.
41 Durand RE, Sutherland RM: Effects of intercellular contact on repair of radiation damage. Exp Cell Res 1972;71:75–80.
42 Olive PL, Durand RE: Drug and radiation resistance in spheroids: Cell contact and kinetics. Cancer Metastasis Rev 1994;13:121–138.
43 Dertinger H, Hulser D: Increased radioresistance of cells in cultured multicell spheroids. I. Dependence of cellular interaction. Radiat Environ Biophys 1981;19:101–107.
44 Luo C, MacPhial SH, Dougherty GJ, Naus CC, Olive PL: Radiation response of connexin43-transfected cells in relation to the 'contact effect'. Exp Cell Res 1997;234:225–232.
45 Franko AJ, Sutherland RM: Oxygen diffusion distance and development of necrosis in multicell spheroids. Radiat Res 1979;79:439–453.
46 Li CK: The glucose distribution in 9L rat brain multicell tumor spheroids and its effect on cell necrosis. Cancer 1982;50:2066–2073.
47 Mueller-Klieser W, Freyer JP, Sutherland RM: Evidence for a major role of glucose in controlling development of necrosis in EMT6/Ro multicell tumor spheroids. Adv Exp Med Biol 1983;159:487–495.
48 Groebe K, Mueller-Klieser W: On the relation between size of necrosis and diameter of tumor spheroids. Int J Radiat Oncol Biol Phys 1996; 34:395–401.
49 Walenta S, Dotsch J, Bourrat-Flock B, Mueller-Klieser W: Size-dependent oxygenation and energy status in multicellular tumor spheroids. Adv Exp Med Biol 1990;277:889–893.
50 Franko AJ, Parliament MB, Allalunis-Turner MJ, Wolokoff BG: Variable presence of hypoxia in M006 human glioma spheroids and in spheroids and xenografts of clonally derived sublines. Br J Cancer 1998;78:1261–1268.
51 Teutsch HF, Goellner A, Mueller-Klieser W: Glucose levels and succinate and lactate dehydrogenase activity in EMT6/Ro tumor spheroids. Eur J Cell Biol 1995;66:302–307.
52 Desbaillets I, Diserens AC, Tribolet N, Hamou MF, Van Meir EG: Upregulation of interleukin 8 by oxygen-deprived cells in glioblastoma suggests a role in leukocyte activation, chemotaxis, and angiogenesis. J Exp Med 1997;186:1201–1212.
53 Dangles V, Femenia F, Laine V, Berthelemy M, Le Rhun D, Poupon MF, Levy D, Schwartz-Cornil I: Two- and three-dimensional cell structures govern epidermal growth factor survival function in human bladder carcinoma cell lines. Cancer Res 1997;57:3360–3364.
54 Frankel A, Buckman R, Kerbel RS: Abrogation of Taxol-induced G2-M arrest and apoptosis in human ovarian cancer cells grown as multicellular tumor spheroids. Cancer Res 1997;57: 2388–2393.
55 Vitale M, Casamassima A, Illario M, Bassi V, Fenzi G, Rossi G: Cell-to-cell contact modulates the expression of the beta 1 family of integrins in primary cultures of thyroid cells. Exp Cell Res 1995;220:124–129.
56 Gehlsen KR, Davis GE, Sriramarao P: Integrin expression in human melanoma cells with differing invasive and metastatic properties. Clin Exp Metastasis 1992;10:111–120.
57 Jones J, Sugiyama M, Speight PM, Watt FM: Restoration of alpha v beta 5 integrin expression in neoplastic keratinocytes results in increased capacity for terminal differentiation and suppression of anchorage-independent growth. Oncogene 1996;12:119–126.
58 Saulnier R, Chan B, Uniyal S, Chan T, Patrzykat A, Elliott BE: The role of integrins and extracellular matrix in anchorage-independent growth of a mammary carcinoma cell line. Cell Mol Biol 1997;43:455–468.
59 Waleh NS, Gallo J, Grant TD, Murphy BJ, Kramer RH, Sutherland RM: Selective down-regulation of integrin receptors in spheroids of squamous cell carcinoma. Cancer Res 1994;54: 838–843.
60 Hauptmann S, Denkert C, Lohrke H, Tietze L, Ott S, Klosterhalfen B, Mittermayer C: Integrin expression on colorectal tumor cells growing as monolayers, as multicellular tumor spheroids, or in nude mice. Int J Cancer 1995;61:819–825.
61 Toole BP: Hyaluronan and its binding proteins, the hyaladherins. Curr Opin Cell Biol 1990;2:839–844.
62 Maurer CA, Freiss H, Kretschmann B, Wildi S, Muller C, Graber H, Schilling M, Buchler MW: Over-expression of ICAM-1, VCAM-1 and ELAM-1 might influence tumor progression in colorectal cancer. Int J Cancer 1998;79:76–81.
63 Ogawa Y, Hirakawa K, Nakata B, Fujihara T, Sawada T, Kato Y, Yoshikawa K, Sowa M: Expression of intercellular adhesion molecule-1 in invasive breast cancer reflects low growth potential, negative lymph node involvement, and good prognosis. Clin Cancer Res 1998;4: 31–36.