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Cellular signaling: aspects for tumor diagnosis and therapy

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Abstract

Cells are organic microsystems with functional compartments interconnected by complex signal chains. Intracellular signaling routes and signal reception from the extracellular environment are characterized by redundancy, i.e., parallel pathways exist. If a cell is exposed to an external "signal input", the signal processing elements within the cell provide a response that will be a pattern of reactions manifest as a metabolic, morphologic or electric "signal output". Cell-chip hybrid structures are miniaturized analytical systems with the capability to monitor such cell responses in real time and under continuous control of the environmental conditions. A system analysis approach gives an idea of how the biological component of these hybrid structures works. This is exemplified by the putative role of the microenvironmental pH as a parameter of the utmost importance for the malignant "mode" of tumor cells, which can be monitored and modeled on such hybrid structures.

Keywords: biohybrid structures; cell metabolism; systems biology; tumor biology.

Cells as signal processors

All living cells, independent of their individual type (e.g., neurons, bone cells, somatic cells, etc.), are extremely complex microstructures composed of different functional subunits at the "nano-size" level. Individual subunits are coupled to each other by biochemical and physical signaling pathways [1]. Thus, processing of these biosignals must be realized within the cell structure. At first glance, the diversity of ultrastructural patterns found in more complex cells appears to be very confusing (Figure 1). During the last few decades, however, analytical techniques such as electron microscopy combined with image analysis have revealed deep insight into the structural organization of cellular pattern and their functional correlates [2]. Other techniques such as analytical light microscopy allow monitoring of the dynamics of cellular signal transduction. As the best-known example, Ca^{2+} chelator dyes such as Fura-2 have revealed the role of receptor-mediated oscillations of intracellular Ca^{2+} as a primary regulation element in cell signaling [3, 4]. Apart from Ca^{2+}, other ion species are now the focus of attention, particularly since transmembrane potentials coupled to such ion gradients can obviously be assigned to pathophysiological cell modes [5]. From an evolutionary point of view, it appears that at the origin of complex cell structures, extra- and intracellular communication was dominated by ion gradients.

Cellular signal processing is non-linear and parallel, thereby involving a high degree of redundancy [1, 6]. The activities of an overwhelmingly complex and dynamically regulated network of signal transmission pathways are controlled by multiple factors. The action of regulatory circuits, cross-talk between pathways, non-linear reaction kinetics of biochemical processes and the compartmentation of cells into many quasi-autonomous but strongly interacting subsystems complicate the understanding and prediction of the outcome of intracellular signaling.

Cells are continuously integrating different sources of chemical and physical signals originating from both internal and external environments. The "output" of this cellular signaling network may be a drastically "amplified" cellular response that is manifest as a trigger for growth and mitosis, the activation of distinct metabolic pathways, the production and release of proteins, a change in cell morphology or motility, the initiation of programmed cell death, or the transmission of another chemical or electrical signal to neighboring cells (Figure 2); all are the result of complex signal evaluation.

One of the most challenging tasks of theoretical biology is to quantitatively describe the mutual interference among all these cellular signaling chains by some abstract model. System biology is usually defined as the study of all elements in a biological system (e.g., a cell) and their relationship to one another in response to perturbations. The ultimate aim of system biology is to develop a mathematical model of the system-level dynamics of such biological systems. Benefits include improved understanding of pathological cellular states, providing therapeutic targets for the treatment of diseases and allowing testing of the effect of drugs *in silico*. The question arises as to whether such a cell *in silico* is relevant to real cells, since the quality of the computer model depends on the quality and completeness of the underlying data collected in many laboratories worldwide. These data include data on the number (concentration) and activity of receptors and enzymes; the concentration and binding kinetics of enzyme substrates and regulating effector substances; and the corresponding functional relationships between the concentration and regulation effects of these effectors. Moreover, the non-linear kinetics of biochemical systems makes it impossible to predict the dynamics of systems over a longer time scale, and such models show a tendency to neglect the memory effects that occur in real cells. Figure

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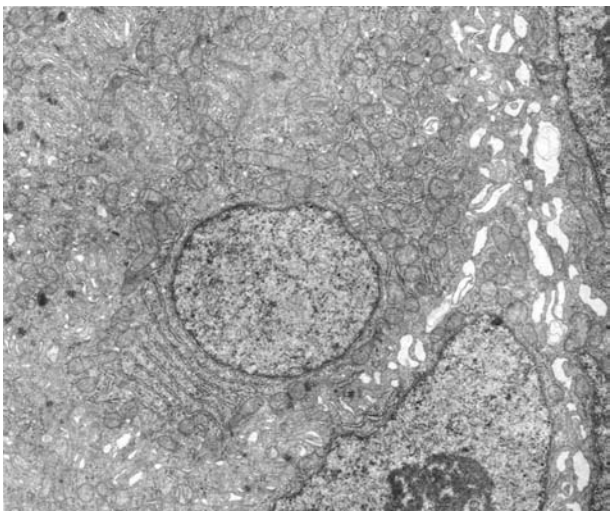

Figure 1 Electron micrograph of an epithelial cell from colon tissue demonstrating the high degree of compartmentation by electroactive membranes and the diversity of ultrastructural patterns.

3 illustrates the parallel computing capabilities that are characteristic for essentially all cell types in comparison with the actual models.

Apart from the biochemical regulation of pathways – e.g., by a fine-tuned balance between phosphorylation and dephosphorylation reactions – highly developed

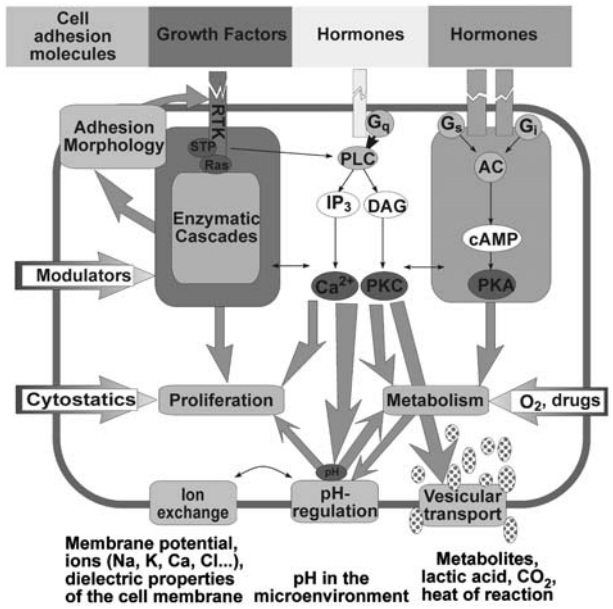

Figure 2 Considerably simplified summary of the mechanisms involved in the cellular processing and transformation of biomolecular and pharmacological signals.
Various growth factors, hormones, and molecules of the extracellular matrix are specifically recognized by plasma membrane receptors. These signals are processed and amplified by the intracellular signal transduction network. Among other signaling cascades, ions exchanged between the intracellular and extracellular space play an important messenger role. These ions modulate the membrane potential and regulate intracellular effectors through cooperative excitation or inhibition. The scheme does not include the large degree of interconnections between the individual signal pathways, nor the great number of positive and negative feedback mechanisms actually regulating the cellular signal network.

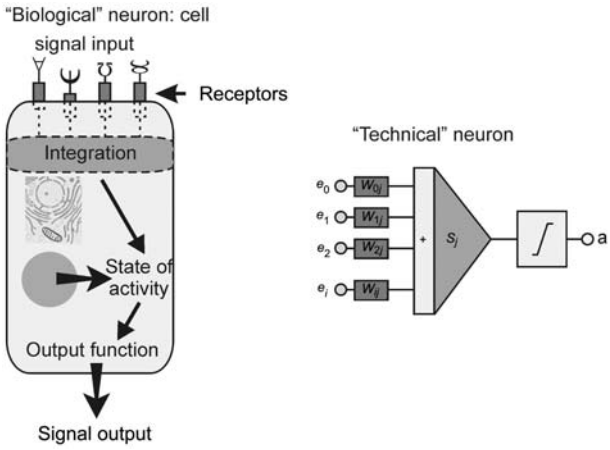

Figure 3 Schematic comparison of signal processing in living cells and technical neurons.
The calculation performance of cells in combination with microelectronic signal transducers allows the development of biohybrid devices. Such devices can be applied to pharmaceutical drug screening, clinical testing and to studies of complex problems in bioinformatics.

mechanisms exist for electrical information processing between different cellular subsystems (see Figure 1). Some of these nanoscale data processing units are primarily controlled by electrochemical gradients. Many examples, such as the organization of the electron transport chains associated with photosynthesis and respiration and membrane-bound signal transduction mechanisms, clearly demonstrate the high degree of nanostructure achieved by cellular systems during evolution, in a way not achievable by technical means until today.

Structured biological modeling

Different tools and approaches for numerical description of cellular signaling pathways have been described. One of them was developed in our own laboratory and is called structured biological modeling (SBM). SBM deals with the problem of how to translate an immense amount of experimental information about a certain biological system into a consistent model that includes its main regulatory processes. Consequently, the central aim of SBM is to determine how a biological system (e.g., the "cell" system) transforms its input (e.g., hormone stimulation of a cell surface receptor) into a specific output (e.g., oscillatory waves of the intracellular free concentration of Ca^{2+} ions, which encodes important messenger information for many intracellular processes). After the integration of pathway information (e.g., from databases) into data flow diagrams and the mathematical interpretation of such diagrams, specifications for a stochastic simulation of the process (e.g., induced Ca^{2+} oscillation) can be fixed. In general, stochastic methods seem to be more appropriate to highly non-linear and multidimensional systems than models using deterministic differential equations. SBM has proved to yield mathematical descriptions of selected cellular subsystems [4]. It provides a tool for the construction of data flow diagrams as the basis for computer simulations of even more com-

plex processes (e.g., malignant transformation of tumor cells), although the predictive value of such simulations is still hampered by the inconsistency and incompleteness of the existing database.

The role of microenvironmental pH as a primary parameter in tumor cell signaling

The treatment of neoplastic transformation of tumor cells in system biology leads to the concept of cellular self-organization. Principally, the function of the "microsystem cell" can, in mathematical terms, be described as a dynamic system. The non-linearity of the system in combination with a permanent energy supply can lead to the formation of quasi-stable modes, which can in turn be macroscopically characterized by suitable order parameters. These quasi-stable modes refer to the robustness of cellular systems, which are both relatively insensitive to alterations of their internal parameters and able to adapt to changes in their environment. Such properties are achieved through feedback, modularity, redundancy and structural stability [8].

A striking common feature of the majority of solid tumors is their deranged metabolism, characterized by an increased rate of glycolysis and extrusion of acid into the microenvironment while maintaining the intracellular pH at a normal or slightly alkaline level. In advanced clinical diagnostics (PET, NMR spectroscopy), this distinct phenotype is already used to classify cancer malignancy or to detect disseminated tumor metastases in the body. From the viewpoint of system biology, the tumor cell has shifted from the "normal" mode into a pathological neoplastic mode. Having undergone this transition, it is extremely robust in terms of its own growth and survival, even under hostile environmental conditions characterized by hypoxia and insufficient supply of nutrients. It appears that the microenvironmental proton concentration is one of the key parameters describing the pathophysiological state of a tumor cell [9–11]. Figure 4 shows experimental evidence indicating that the low pH surrounding tumor cells also seems to counteract the activity of the cellular immune system, which is frequently found to be colocalized with tumor cells in cancerous tissues [12].

Taking into account the intracellular proton concentration, kept low by the hyperactivity of proton transporters and thus biased to a level that drives the cell into permanent and uncontrolled proliferation, a "messenger" function can be attributed to the intratumoral pH value itself. On the one hand, SBM can be used to set up data-flow diagrams to integrate existing experimental data into consistent theoretical models as the basis for subsequent stochastic simulation of tumor growth and interaction between tumor cells and the immune system. This approach reveals the implications of a deranged tumor cell metabolism and acidic microenvironment for the progression of solid tumors [13]. On the other hand, given the importance of microenvironmental concentrations of protons, oxygen, and limiting nutrients for the pathological state of tumor cells, suitable techniques must be used for the practical *in vitro* on-line monitoring of these substances and of the system-level operation of the

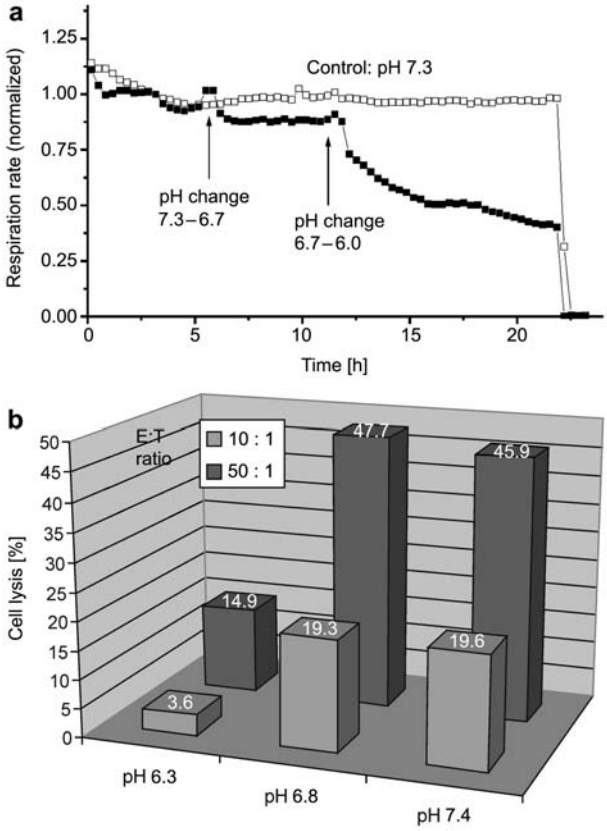

Figure 4 The respiratory (a) and cytolytic activity (b) of lymphocytes derived from peripheral human blood depends on the microenvironmental pH.
The graphs in (a) were obtained with biohybrid devices described in the text. Decreasing the extracellular pH to values below 6.7, which are frequently found in solid tumors, leads to strong inhibition of respiration. Under these conditions, the cytolytic activity is also impaired, given for different ratios of IL-2-activated peripheral blood lymphocyte effector cells to tumor target cells, the "E/T ratio".

cells. The aim would be to provide an additional tool for pathological diagnostics, i.e., to analyze the deranged metabolism of tumor cells derived from biopsy material. Moreover, experimental modeling of *in vivo* tumor conditions (with and without therapeutic intervention) would allow an improvement of cancer therapy. Promising candidates for such monitoring are so-called biohybrid structures.

Response profiles of cells monitored with biohybrid structures

With the parallel signal processing capabilities of cells in mind, it is clear that the "reductionist" paradigm of molecular biology (e.g., attempting to switch a cellular operation by manipulating a single or a few genetic elements) could not be successful. Recent advances in the analysis of gene expression and protein patterns provide access to certain aspects of system biology complexity, but there is currently no direct link between such compositional data and the dynamic metabolic and physiological aspects of cellular systems.

A practical approach to cell system biology could be the design of (primary) human cell models (including the

incorporation of multiple cell types) and the combination of such models with automated assays measuring disease-relevant cellular responses, e.g., to a pharmacological or environmental input. This concept avoids the need for complex *in silico* models, the application of which will be far in the future [14]. Certainly, this approach is revealing, even in the absence of detailed knowledge about involved biochemical targets, because many pathways are activated in parallel and the resulting response pattern can be analyzed. The readout of the response profile generated serves as a signature of all input signaling in the cell system investigated. This may be the measurement of a set of proteins or a combination of extracellular parameters that can be monitored dynamically with "biohybrid devices". These devices are functional units formed by cell cultures growing directly on microelectronic sensor chips whilst embedded in a fluidic life-support system mimicking the usual cellular environment.

In contrast to the powerful techniques of analytical microscopy and image analysis or NMR spectroscopy, biohybrid devices are usually small and easy-to-handle devices with the capability to monitor dynamic alterations occurring in the sample (Figure 5). The direct and extracellular mode of measurement avoids the need for any labeling steps. Most importantly, the measurement itself does not disturb cellular behavior. Owing to the small size of the sensor chips (typically with a cell growth area of approx. 50 mm^2) the cell number required is within the range of only 10^4–10^5. Complex primary cell systems, such as explants from human tumors (incorporating an "ecosystem" of tumor cells together with various types of stroma cells), can be cultured on the chip surface.

Details of the different microsensor elements (potentiometric, amperometric and impedimetric structures) have been published elsewhere [15, 16]. Briefly, the metabolic activity of cells is measured with extracellular sensors for pH and pO_2. The extracellular pH is a parameter reflecting the extrusion of acidic metabolic waste products across the cell membrane. It is significantly increased in most tumor cells. The level of dissolved oxygen (pO_2) can be used as an indicator of the activity of the mitochondrial respiratory chain. Impedimetric electrode structures may detect adherent cell growth and subtle changes in cell morphology. Metabolic rates and the structure of the cytoskeleton are very likely to be affected by any pharmacological or environmental signal inputs. The addition of agonists to different types of cellular receptors is known to affect cell metabolism and cell morphology in a very specific, dynamic manner, depending on the pathways being activated [17–19].

As for almost every biological experiment, statistical treatment of results is mandatory. In the case of biohybrid structures, there is a demand for robust, automated assays that incorporate modeling of complex cellular microenvironments [20]. Therefore, we started to develop medium-density plates with multiparameter glass sensor chips. These plates are operated within climate boxes and connected to pipetting robots for liquid handling and to microscope platforms for additional cell imaging and optical readout (Figure 6).

Special attention has to be directed to a practical solution for electric contacts and to appropriate pre-processing and compression of the data obtained. One of the most important benefits of complementary metal oxide semiconductor (CMOS) technology is the possibility of on-chip circuitry for signal amplification, data analysis and sensor self-testing. On-chip sensor multiplexing would be a precondition for the construction of 96- or 384- multiwell arrays, since otherwise the number of electric connections necessary becomes unmanageable. Moreover, arrays of sensors can be formed on individual chips. An example would be an array of ion-selective field effect transistors (ISFETs) for the measurement of pH gradients directly beneath growing cells with high local resolution.

The information gained from such multisensor arrays does not depend on the characteristics of a single sensor of this array, but rather on the whole signal pattern, which requires another type of biosignal processing. Pattern recognition is a general problem in biological and technical applications such as image analysis, automatic speech recognition, and optical character recognition.

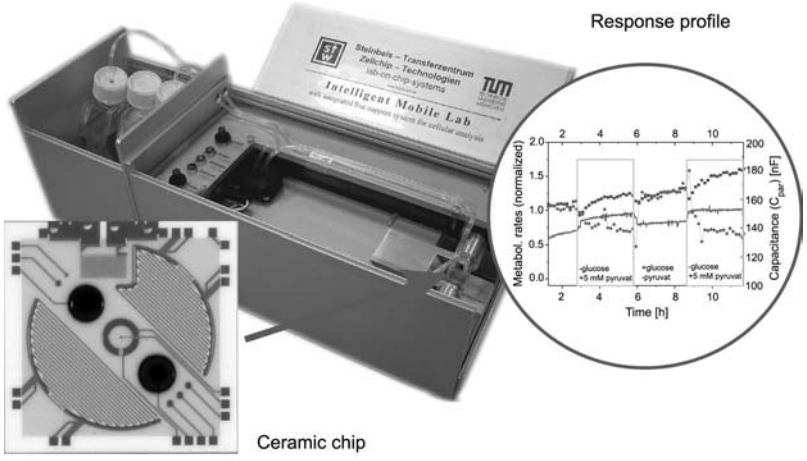

Figure 5 The "intelligent mobile lab": compact and portable device integrating microfluidics and electronics for single cell chips. A chip for cultivation of cells with sensors for pH, dissolved oxygen and electric impedance is shown. Extracellular "output" signals (i.e., acidification, cell respiration and morphological changes) are converted, preprocessed, analyzed, stored as analyzed data ("response profile") and displayed on an external computer system.

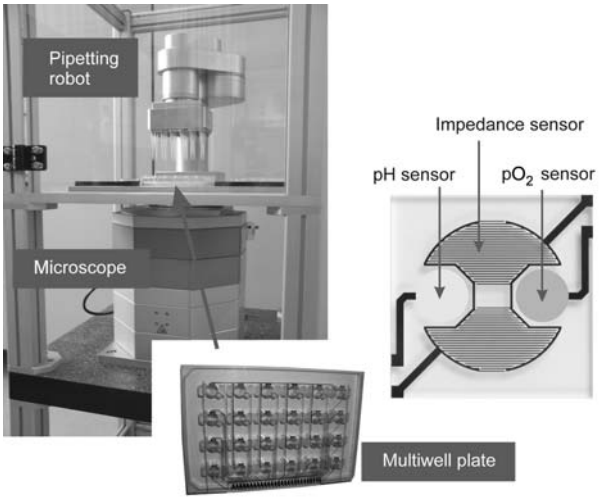

Figure 6 Novel automated assay system using extracellular sensor technology.
The system incorporates a climate box to maintain sterility, temperature and humidity, a liquid handling robot, and a digital inverted microscope (TILL-Photonics GmbH, Gräfelfing, Germany). If the microscope is omitted, optical sensors for pH and dissolved oxygen can be used on the glass chips instead of electric sensors. Sensor and image data are processed by an external computer.

Sophisticated techniques exist to solve this problem, e.g., on the basis of neural networks or hidden Markov processes; these are needed to analyze the patterns generated upon the application of different signal inputs to complex cell systems.

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