Impedance-based analysis applied as a dedicated technique to characterize efficacy of novel antitumour compounds

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Evaluation of candidate molecules as dedicated antitumour compounds is a complex, multistep process which prefers high throughput and real-time cell physiological assays. Application of impedimetry-based systems (ECIS, xCELLigence etc.) in combination with other cell physiology/molecular biology HCS platforms (e.g. CellDiscoverer7 – Zeiss) provides the possibility to perform large number of accurate measurements required by pharmaceutical research.
In the last decade, hundreds of drug candidates (pure active ingredients and conjugates with targeting moiety and delivery units) have been screened in our laboratory.
(i) In these investigations the most frequently tested drug-carrier constructs were belonging to peptide- or protein-based structures: daunomycin- (Dau), doxorubicin- (Dox) or methotrexate- (Mtx) GnRH conjugates; while there were others selected by phage display technique as well as another list of ferrocene containing hybrids, cinchona alkaloids, tamoxifens and TIC-10.
(ii) The most often used target cells were: human pancreas ductal carcinoma (PANC-1), human acute monocytic leukemia (Mono Mac-6); human melanoma (A2058 and HT168-M1); human colorectal carcinoma (COLO-205) and choriocarcinoma (BeWo) cell lines.
(iii) While the monitored cell physiological characteristics (proliferation, cell adhesion, migration) as well as apoptotic characteristics were analyzed by impedimetry and colorimetric assays (e.g. AlamarBlue). The registered antitumor effects were confirmed by computer assisted analysis of morphometry and migratory behaviour in holographic microscopy.
In impedimetry-based cytotoxicity/viability assays (e.g. in PANC-1) showed that in the group of peptide-based compounds (i) some conjugates containing Dau (e.g. KK031), selected by phage display technique, expressed the highest cytotoxic effect (viability 8.6 % at 10^{-5} M); (ii) the neurotensin-based conjugates were also effective (viability 4.6 %) in tumor cells, nevertheless in these type of constructs the dual targeting or formation of bifunctional conjugates could also enhance the cytotoxicity of the antitumour compound. In the group of non-peptide-type ingredients (iii) cinchona alkaloids and TIC-10 derivatives proved to be the most effective (IC50 = 3E-6 - 6E-6 and IC50 = 7E-7 - 3E-6 respectively); (iv) formation of ferrocene hybrids of cinchona alkaloids resulted also enhanced cytotoxic effects both in PAN-1 and COLO-205 cells; while (v) the increased cytotoxicity of the halogenated TIC-10 vs. the parent molecule was also detected (IC50 = 3.4E-7 vs. IC50 = 1,7E-6).
Results presented above as well as series of data gained by other probes of cell physiology (to be presented in the oral presentation) support our opinion that impedimetry is a strong arm of experimental screening and complex evaluation of a wide range of antitumour compounds.

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