Magnetic Resonance Imaging and Ultrasonography of Chicken Egg Tumor Model

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Introduction

Some forms of malignant tumors, such as melanomas, are extremely aggressive and rapidly fatal. Preclinical studies still lack of effective and reasonably priced methods and models for testing possible drug target response, i.e. following the tumor growth without terminating tumors for histological analysis in each stage of its development. Different tumor cell lines may bring up either flat or round tumors on egg chorioallantoic membrane (CAM). Egg models are widely used in preclinical science as they are cheap, not labor-intensive and they are considered as non-animal experiments which do not require special permission for experimental animal work in most of the countries. However, imaging of egg is technically demanding in use due to the egg shell structure and mobility of yolk and embryo. We used ultrasonography (USG) and MR-imaging to categorize tumor growth noninvasively during 10 days. MRI can be used for tumor size monitoring after the tumor size has reached a few mm, before that partial voluming effects would reduce the reliability of MRI. Main purpose of this study is to combine advantages of egg models in oncobiology and imaging techniques for creating routines to serve this model for oncobiological applications.

Methods

The mice melanoma (B16F0) model was used. Briefly, 30 chicken eggs at age 8 days (13 days to hesting) were taken, air bubble of egg was sucked out to divide CAM from shell and approximately 7x7 mm window was drilled to the side of egg. Then ~7000 melanoma cells were implanted to the chorioallantoic membrane and window was closed by adhesive tape. These eggs were imaged 6, 8 and 10 days later by using clinical ultrasonography imaging device (LOGIQ 100 MP, GE Healthcare/GE Medical Systems, gynaecologic detector). Detector was inserted to an egg through the artificial window while microbiological contamination of egg was avoided by sterile polyethylene membrane and 0.9% sodium chloride solution covering the yolk and tumor(s) during imaging session. In addition, 3 eggs were imaged 10 days after cell implantation by using horizontal 4.7 T magnet (Varian ^{UNITY}INOVA). Litz-type volume coil was used in transmitter-receiver mode. To avoid the movement artifacts, the eggs were cooled down to +14 degrees of Celsius using a handmade egg cooler. T_2-weighetd (TE=40 ms, TR=3000 ms) spin echo imaging was performed (data matrix 512x256, 15 slices, slice thickness 1mm). After the imaging session the eggs were cooled down until +4 degrees by Celsius to sacrifice these for histology and the tumor size measurements by micrometer.

Results and Discussion

Mice melanomas B16F0 started to grew in 24 of 30 eggs (80%). Each tumor differs from others by the size, shape, vascularization and by CAM blood vessel aberration response to tumor. *In vivo* USG managed to detect reliably tumor size during the last week of tumor growth period (FIG 1). Since the CAM is in the end of its life span days before hesting, tumors started to suffer the lack of blood circulation and MRI showed inhomogenic areas in all studied tumors (example egg #10 on FIG 2). Contrary, the USG did not show any inhomogeneities since the size of the tumors was measurable with clinical ultrasound imaging equipments starting at 2 mm diameter, as it gave hardly recognizable images from tumors. *In ovo* tumors showed transformed radial orientation of blood vessels in CAM, but in the latter time point it was not visible anymore. Besides the ongoing atrophy-induced malfunction of CAM vascularity and bleeding was common (FIG 3). The movement artifacts of chicken embryo *in vivo* (*in ovo*) imaging can be avoided by cooling down the egg temperature, but it suppresses blood circulation which may cause problems in e.g. MR-angiography. MRI enables also monitoring of the developmental stages of chicken embryo itself until hesting.

Conclusions

Here we present one cost-effective tumor model combined with MRI and USG. *In ovo* i.e egg models are very cheap for testing the effect of new drug candidates and allow follow-up of tumor development. In addition, *in ovo* models stimulate collaboration between institutions and countries due to simple legislational aspects. Furthermore, experiments with the used cell line (mice melanoma B16F0) can be continued in mammals for testing drug effectiveness in more complex ways. All commonly exploited imaging methods (MRI, USG etc) give valuable information for screenings. In conclusion, in ovo experiments can be suggested as a first option for many oncobiological studies as they do not require any specific skills, permissions or conditions and they help to plan forthcoming experiments in low budget. Naturally, the list of tumor cell lines available extends fast and those cell lines should be tested also for *in ovo* tumor imaging models.

Figure 1: Egg #10 with melanoma (diameter 5 mm) detected by ultrasonography 8 days after implantation
Figure 2: Egg #10 two melanomas visible by T_2-weighted MRI 10 days after implantation
Figure 3: Egg #10 microphotograph, two bigger and multiple smaller melanomas laying on chorioallantoic membrane (imaged immediately after MRI session)