CHAPTER V
DISCUSSION

Some parts of the discussion have been made in the relevant results (Chapter IV). General considerations were added in this chapter on the 2 topics; (i) the biology and the basic characteristics of the cell lines and (ii) the cytotoxicity assay of the plants crude extracts to the cell lines.

5.1. The biology and basic characteristics of the AMC-K46.

Human amniocytes have been used for prenatal diagnosis and reported with effective karyotyping analysis (Chaabouni *et al.*, 2001). The cells received from amniocentesis and could be developed as a continuous cell line after a period of *in vitro* cultivation (Wisadkeaw, 2004; Sangngam, 2005). Spontaneous transformation in the cultured human amniocytes was clearly demonstrated by Walen (2002). This phenomenon is thought to be happened just the same in the AMC-K46. However, no clear evidence have been observed to support with Walen (2002) on this research. The three morphological cell types observed on AMC-K46 have not yet been proven so far, for any specific origin. However, the giant cells, the polyploidy cells, should be the key group of cells causing the neoplasia or the immortalization of the cell line (Levan and Biesele, 1958; Duesberg *et al.*, 2000). The heterogeneity of the cell line (several cell types included in the culture) may be the important factor affecting the bioassay. The different cell types expressed with different capacity of responsiveness to the active agent from the extracts (Freshney, 2000).

The AMC-K46 exhibited 2 phases of growth pattern with the seeding size of $2x10^4$ cells/cm^2. This seeding concentration cause the cells grew faster than that mentioned by Wisadkeaw (2004), although the generation time still the same. The confluent density was considered to be as lower than those reported by Wisadkaew (2004) and Sangngam (2005). This probably due to the unstable of the cell differentiation between the generations of the cell lines (Albert *et al.*, 1994). Furthermore, the technical limitations such as the cells counting under the micrometer OMG-1/100 would probably the addition of numerical errors.

ลิขสิทธิ์มหาวิทยาลัยเชียงใหม่
Copyright© by Chiang Mai University
All rights reserved

The distributions of the total chromosome number pattern of the untreated AMC-K46 (46-136) differed from that reported by Wisadkeaw (2004) (46-69). This might be the sign of the phases of differentiation of the cells along their continuous generations (Walen, 2002). The study of mosaicism in the chromosome distribution and aberration requires the advanced technique such as FISH or SKY (Mitchell, 2000). The expertise, skill and endurance is seems to be very important for such a task. The study on the chromosome of more than 100 metaphases, one by one, emphasizes the procedures with quite tedious and time comsuming.

There was no previous report known to date using the amniotic fluid cell lines in a panel of screening, neither for cytotoxic nor genotoxic agent from natural products. Such the screenings have been done popularly by the "micronucleus assay" using the cells from bone marrow of laboratory animal (Hu *et al.*, 2005; Çelik *et al.*, 2005). 3T3 (the mouse fibroblast cell line) have been used widely as the model for the cytotoxicity testing for the cosmetic product (Chew *et al.*, 2000). AMC-K46 responded to the SDS with the MTT_{50} (71.3 μg/ml) very similar to that of 3T3 (70 μg/ml). This is quite a good tendency of the AMC-K46 (or any other amniotic fluid cell lines) to be used as an embryonic human epithelial cells model for the cosmetic cytotoxic screening in the future.

5.2 The cytotoxicity assay of the plants crude extract to the cell lines.

The crude extract from the 3 plants, *A. reticulata* (young fruit), *A. squamosa* (young fruit) and *M. fruticosum* (leaves), exhibited the effective potent of selectivity to the two cell lines. Acetogenin and kaurane diterpenoids would be the main active agents with highly selective to the two cell lines (Chang, 1993, Wu *et al.*, 1996, Yu *et al.*, 1997, Chang *et al.*, 1998 and Yuan *et al.*, 1998). The other alkaloids or glycosides would also probably be the active agents against the cells (Leboeuf *et al.*, 1982).

Consider on the P.D.M.I., the exposed cells to the crude extracts from *A. reticulata* and *A. squamosa*, which were higher than the unexposed cells (control groups) (see 4.5.3.1). The acetogenin and liriodinine (topoisomerase II inhibitor) would be the effective compounds to the cells (Woo *et al.*, 1997 and Alali *et al.*, 1999). The cause of slightly increasing of the percentage of the chromosome

ลิขสิทธิ์มหาวิทยาลัยเชียงใหม่
Copyright © by Chiang Mai University
All rights reserved

aberration may be possibly from the genetic instability due to the differentiation of the cells (Walen, 2002). The chromatid type of aberrations observed in all the cells leaded to the conclusion of the effect to the M phase of cell cycle (Mitchell, 2000).

The rounding up of both cell lines with dark blue nucleus may be from the chromatin condensation in the death process (Ebert *et al.*, 2005). The cells may continuously death by the delay effect which stimulated by the extracts. These effects needed to be studied further using the cell death detection techniques such as, DNA ladders or Caspase detection etc.

The young fruit of *A. reticulata* exhibited the highest effect to the cells in all aspects of this study. As far as known to date, this is the first record of the young fruit of the annonaceous plant to be extracted and studied. However, as this was a preliminary screening, further studies with the purification of the active compounds would be of great interest.

ลิขสิทธิ์มหาวิทยาลัยเชียงใหม่
Copyright© by Chiang Mai University
All rights reserved